THE ROLE OF SELECTION WITHIN PLANT COMMUNITIES FOR ECOSYSTEM FUNCTIONING

Dissertation

zur

Erlangung der naturwissenschaftlichen Doktorwürde

(Dr. sc. nat.)

vorgelegt der

Mathematisch-naturwissenschaftlichen Fakultät

der

Universität Zürich

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Zürich, 2014

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“If I have seen further it is by standing on the shoulders of giants”

Letter from Isaac Newton to Robert Hooke, 5 February 1676, as transcribed in Jean-Pierre Maury (1992) Newton: Understanding the Cosmos, New Horizons

Summary

Summary

In biodiversity experiments, the magnitude of the positive effects of species diversity on ecosystem functioning increases over time, yet it remains unclear which mechanisms drive this increase. The strengthening of the biodiversity effect on performance has been interpreted as a potential consequence of increasing complementary resource use between, or pathogen regulation of, species or plant functional groups. However, such an increase in the biodiversity effect could also be due to selection for increased combining ability via division of labor in high-diversity plant communities (**Chapter 1**). A potential mechanism for increased division of labor could be selection for increased functional trait differences between species. I tested this novel hypothesis by comparing the performance of plants in monocultures vs. mixtures collected after 8 years in plots of a grassland biodiversity experiment containing single species (monocultures) or mixtures of species. I found that mixtures had the highest performance if they contained plants collected from mixtures and monocultures had the highest performance if they contained plants collected from monocultures, indicating that selection for mixture or monoculture types had occurred in the biodiversity experiment. These findings are consistent with the hypothesis that selection for mixture types with increased division of labor via larger functional trait differences occurs in mixed-species communities. The results of this experiment offer a novel mechanism explaining the increase in biodiversity effects over time in experimental mixed grasslands

Plant–plant and plant–soil interactions are key for the maintenance of species diversity in grassland ecosystems and the ecosystem services they provide. Increased complementarity among species is currently thought to be the main factor and recently plant–soil feedbacks have been proposed to account for such increasing complementarity effects. I present a novel hypothesis, that such increasing complementarity effects are driven by soil legacy, the co-evolution of soil biota, interactively with plant legacy, plants selected in experimental mixtures or monocultures of grassland plant species over eight years (**Chapter 2**). I tested the

interaction of soil legacy with plant legacy on complementarity effects. Furthermore, I examined the effects of plant legacy and soil legacy on weed establishment with experimental communities. I show that plant and soil legacy interactively modified the biodiversity effects. In particular, complementarity effects were largest for potted communities assembled from progeny of plants selected in mixture and grown on soil with inoculum from mixture plots.

Finally, I test whether metabolic changes occur in response to selection in a community with a selection history of either monoculture or mixed species diversity over 8 years in the Jena Experiment, Germany (**Chapter 3)**. I used Fourier transform infrared spectroscopy (FTIR), with chemo-metrical data treatment, to discriminate between leaf samples of individuals from either monoculture communities or mixed-species communities. I tested whether alterations in biochemical composition occurred in plants selected in monoculture and mixed-species communities. Using the collected FTIR spectra, replicate samples of leaf material from individuals selected either in monoculture or mixed-species communities were analyzed using multivariate procedures (LDA, NMDS, CVA and cluster analysis). Plant individuals were correctly classified as either from monoculture or mixed species selection history. Furthermore, using the corrected spectra, I determined that the greatest statistical distance was between the two groups. I demonstrate that alterations in biochemical composition change the fingerprint of plant individuals of eight species selected for in monocultures and in mixtures over 8 years indicating metabolic changes.

The results of this study offer novel insights into the increase in biodiversity effects over time in experimental mixed grasslands indicating that species diversity may influence selection processes within plant communities. Furthermore these results deepen our understanding of the mechanisms governing plant coexistence and of the long term impacts of biodiversity loss.

Zusammenfassung

Bei Biodiversitätsexperimenten mit Wiesenpflanzen nimmt der positive Effekt der zunehmenden Arten- und Funktionsvielfalt auf den primären Ertrag mit der Zeit zu. Eine mögliche Erklärung dafür ist, dass vielfältigere Pflanzengemeinschaften aufgrund komplementärer Eigenschaften die vorhandenen Ressourcen über die Zeit effizienter nutzen können oder dass Krankheitserreger die Arten regulieren. Allerdings könnten solche Zunahmen auch durch Selektion von Individuen mit erhöhter Ressourcenkomplementarität durch Merkmalsunterschiede zwischen Pflanzenarten in vielfältigen Pflanzengemeinschaften zustande kommen, was nach und nach zu erhöhter Arbeitsteilung führen würde (**Kapitel 1**). Diese neuartige Hypothese testete ich, indem ich Leistung und Morphologie von Pflanzen unter Mono- und Mischkulturbedingungen verglich. Die dafür benutzten Pflanzen stammten aus einem acht Jahre alten Biodiversitätsexperiment, in welchem Monokulturen oder Mischkulturen mit einer oder vier funktionellen Gruppen (Gräser, Leguminosen, hochgewachsene Kräuter, niedere Kräuter) angepflanzt worden waren. Ich stellte fest, dass neue Mischkulturen den grössten Ertrag hatten, wenn diese Pflanzen aus alten Mischkulturen enthielten, und dass neue Monokulturen den grössten Ertrag hatten, wenn diese Pflanzen aus alten Monokulturen enthielten. Dies weist darauf hin, dass während des Biodiversitätsexperimentes eine Selektion für Misch- oder Monokulturtypen erfolgte. Meine Resultate unterstützen somit die Hypothese, dass in Mischkulturen mit verschiedenen Arten eine Selektion für Pflanzentypen mit erhöhter zwischenartlicher Kombinationsfähigkeit auftritt. Sie liefern damit neue, spannende Erkenntnisse zum Mechanismus, der in Experimenten mit unterschiedlich diversen Wiesenflächen im Lauf der Zeit zu erhöhten Biodiversitätseffekten führt.

Interaktionen zwischen Pflanzen und zwischen Pflanzen und Bodenorganismen sind der Schlüssel für die Erhaltung der Artenvielfalt in Wiesenökosystemen sowie deren

Ökosystemdienstleistungen. Der positive Effekt der Biodiversität auf die Primärproduktion steigt mit der Zeit an. Es war bisher allerdings unklar, welche Mechanismen dafür verantwortlich sind. Zur Zeit erachtet man die erhöhte Komplementarität zwischen Arten als Hauptfaktor für den steigenden Biodiversitätseffekt; als weiterer Faktor wurden in letzter Zeit Interaktionen zwischen Pflanzen und Bodenorganismen vorgeschlagen. Ich stellte die neue Hypothese auf, dass die steigenden Komplementaritätseffekte gemeinsam gesteuert werden durch Pflanzen–Boden-Rückwirkungen, d.h. die Vorgeschichte des Bodens, und durch Selektion von Pflanzen, die sich an ihre Pflanzengemeinschaft angepasst haben, also die Vorgeschichte der Pflanzen (**Kapitel 2**). Ich testete die Auswirkungen solcher Pflanzen–Boden-Rückwirkungen auf die Komplementarität zwischen Pflanzen, die während acht Jahren in experimentellen Wiesenmischungen oder Monokulturen selektioniert worden waren. Weiter untersuchte ich die Auswirkungen dieser Selektion sowie der Pflanzen–Boden-Rückwirkungen auf das Aufkommen von Unkräutern in neu zusammengestellten experimentellen Gemeinschaften. Ich konnte zeigen, dass die Selektion für steigende Komplementarität mit der Selektion für reduzierte Pflanzen–Boden-Rückwirkungen einhergeht. Das Ergebnis dieser Studie deutet darauf hin, dass Pflanzen–Boden-Rückwirkungen die Komplementarität in Gemeinschaften mit einer hohen Pflanzenvielfalt über längere Zeit fördern.

Schliesslich testete ich auch, ob die über acht Jahre in Monokulturen oder Mischungen selektionierten Pflanzen sich auch in ihrem metabolischen Profil unterschieden (**Kapitel 3**). Hierzu verwendete ich Fourier-Transformations-Infrarotspektroskopie (FTIR) mit Blattmaterial. Die gesammelten FTIR Spektren wertete ich mit multivarianten Verfahren aus. In Cluster- und Diskriminanzanalysen konnte ich Pflanzenindividuen unterschiedlicher Selektionsgeschichte nach ihrer Herkunft aus Mono- oder Mischkulturen korrekt klassifizieren.

Die Resultate dieser Studie vermitteln neue Einsichten in die beobachteten Biodiversitätseffekte in experimentellen Wiesenmischungen. Diese deuten darauf hin, dass die Diversität von Arten die Selektion innerhalb von Pflanzengemeinschaften beeinflusst. Dies führt zu neuen Erkenntnissen über die Koexistenz von Pflanzen und vertieft unser Verständnis für die Auswirkungen des Verlustes der Biodiversität.

Introduction

General Introduction

Biodiversity and Ecosystem Functioning

At the Rio de Janeiro Earth Summit in 1992 the recent unprecedented loss of biodiversity was formally recognized for the first time as equivalent to a sixth mass extinction (Totten et al. 2003, Wake and Vredenburg 2008). Over the last 25 years the focus of ecology has therefore shifted toward how species diversity functions within communities by disentangling species interactions to ascertain the consequences of the current and predicted losses of biodiversity. Furthermore ecologists expanded the focus of their research to include the biotic feedback of biodiversity on ecosystem processes (Schulze and Mooney 1993) and functioning (Cardinale et al. 2012, Naeem et al. 2012). Scientists have addressed this topic by means of biodiversity experiments in which species richness is manipulated by either addition or subtraction of species. The initial experimental work focused predominantly on grassland plant communities; such as the Ecotron experiment in England (http://www3.imperial.ac.uk/cpb/history/theecotron/virtualecotron1), the BioDEPTH experiment across Europe(http://forest.bio.ic.ac.uk/cpb/cpb/biodepth/contents.html), a series of experiments at the Cedar Creek Biological Preserve in Minnesota, U.S.A.(http://www.cbs.umn.edu/explore/cedarcreek), and the Jena Experiment, Germany (http://www.the-jena-experiment.de/). Using such experiments as model systems, ecologists could confirm the prediction that increased species richness generally increases ecosystem functioning, typically measured as primary productivity (Balvanera et al. 2006, Cardinale et al. 2006, Isbell et al. 2011, Cardinale et al. 2012). Moreover, the strength of the diversity–productivity relationship has been observed to increase over time (Tilman et al. 2006, Reich et al. 2012).

The observation that plant productivity is greater in species mixtures was initially mentioned by Darwin in 1859, when he hypothesized that such an effect was due to

ecological differences between species in mixtures (Hector and Hooper 2002, Hooper et al. 2005). To date, the positive effect of biodiversity on ecosystem functioning is similarly interpreted as a potential result of niche segregation allowing for complementarity resource use among species (Savage 1958, Case and Gilpin 1974, Silvertown and Law 1987, Silvertown 2004).

Whereas biodiversity experiments so far have focused almost entirely on interactions within the focal trophic level, the role of consumers and pathogens in regulating the number and abundance of species is increasingly being recognized as potentially playing a large role in determining biodiversity effects on ecosystem functioning (Duffy et al. 2007, Long et al. 2007, Eisenhauer 2012). The future of biodiversity research lies therefore in not just focusing on plant–plant interactions, but also plant–pathogen and plant–herbivore interactions.

Niche Theory and Competition

Niche theory attempts to explain how competing species within an ecosystem may coexist and positively impact ecosystem functioning. During the course of the last century niche theory has been the topic of much discussion among ecologists (Warner and Chesson 1985, Hubbell 2005, Adler et al. 2007) despite being widely accepted. The term niche was first used in 1917 referring to the habitat in which a species lives (Grinnell 1917) and was elaborated to include the role a species plays in the habitat (Elton 1927). Niche theory was later expanded to include the idea of species sharing different niches within the same habitat or biotope space, linking the fitness of individuals to their environment (Hutchinson 1957). The ecological niche of a species furthermore refers to the total resource requirements and conditions that determine the range of a species and its abundance within that range (Pidwirny 2006). In the absence of competition from other species a target species could potentially use a large range of resources and accept a range of abiotic conditions, the fundamental niche. In the context of competition and species interactions, however, a species fundamental niche is

reduced to a realized niche by limiting factors such as the presence of competing species in an environment (Severtsov 2004).

Competition is therefore frequently considered to be the most important species interaction for species co-existence within a community (Gause 1934). Competition can occur between individuals of the same species in intraspecific competition and competition can occur between different species, interspecific competition. The competitive exclusion principle proposed that for species to coexist they must have different niches for if the niches of two species would completely overlap only one species could survive interspecific competition (Gause and Witt 1935). Grime proposed that an organism that is a strong competitor for one resource would be a strong competitor for all resources and focused on the role of the environment as the determinant of competition between species (Grime 1979). In contrast, Tilman (1985) suggested that it is rather the difference in ability of species to grow at various levels of two or more resources that could explain species coexistence and competition among species may be mediated by a trade-off of particular functional abilities of a species. For instance, a trade-off of resource allocation occurs if a species has a strong competitive ability for one resource at the cost for that species to have a weak competitive ability for another resource. The focus on the mechanisms for species competition and coexistence between plants has therefore included the role of competition for resources (Tilman 1985). More recently it has been demonstrated, in experimental plant communities, that plants can be differentiated across environmental niche axes suggesting differential resource use (Silvertown 2004). Partitioning in the use of soil nutrients is known to occur among plant species (McKane et al. 2002, Roscher et al. 2008) indicating that coexisting species can indeed be complementary in their resource uptake.

Niche differences between species can therefore decrease the strength of interspecific competition relative to intraspecific competition (Chesson 2000). Thus, selection in high-

diversity communities with high interspecific competition can be expected to favor genotypes with more distinct niches between species, reduced interspecific competition and thus increased combining ability. Reduced interspecific competition could also result from extension of the total community niche (Salles et al. 2009) in addition to, or instead of, finer division of currently used resources. The notion of diversity as a driver of plant population differentiation has been suggested in theory (Vellend and Geber 2005) and a field study has indicated that differential selection for monoculture and mixture types in grassland species may occur (Lipowsky et al. 2011), supporting the proposal that local evolutionary changes arise from selection through competition (Taylor and Aarssen 1990). The positive effect of interspecific combining ability on biomass production can thus potentially explain the increase in biodiversity net and complementarity effects observed in biodiversity experiments (Cardinale et al. 2007, Reich et al. 2012).

Reduced interspecific competition through niche differentiation could therefore lead to complementarity effects among species. Such resource-use complementarity among species is the mechanism on which the general hypothesis for biodiversity effects is founded (Hector 1998, Loreau and Hector 2001, Tilman et al. 2001, HilleRisLambers et al. 2004, Schnitzer et al. 2011). Increased complementarity between species may explain the increase in ecosystem functioning and stability within plant communities with increasing specie richness (Tilman et al. 2001, HilleRisLambers et al. 2004, Schnitzer et al. 2011, de Mazancourt et al. 2013), an effect that often becomes stronger over time in grassland experiments (Tilman et al. 2006). Alternatively, the increased probability of sampling a species with a large contribution to ecosystem functioning in species-rich communities, the so-called selection effects, may also explain positive biodiversity effects on ecosystem functioning (Hector 1998, Loreau and Hector 2001).

Division of Labor

Resource use complementarity could be likened to the concept of division of labor accepted in economics (Lloyd and Dicken 1972) and in social animal communities (Brandão 1978, Ghiselin 1978, Wilson 1978). The premise of division of labor is the specialization of and cooperation between individuals thereby increasing performance of the community as a whole (Lloyd and Dicken 1972, Bloom et al. 1985). A community's performance may be increased by resource use specialization or by trading surpluses (Blau 1970, Lloyd and Dicken 1972). In plants the concept of division of labor has been accepted in clonal plants which are able to exploit resources through specialization and resource sharing (Alpert and Mooney 1986, Stuefer et al. 1994, Stuefer et al. 1996). It is conceivable that a division of labor within grassland plant communities occurs as different species within the community exploit complementary niche space benefitting the performance of the community as a whole. Such division of labor in grassland plant communities may in addition be enhanced by plant–soil feedbacks. As an example, some plants associate with mutualistic soil biota which have been shown to benefit the plant community by increasing diversity and productivity (van der Heijden et al. 1998, Bauer et al. 2012).

Plant–Soil Feedbacks

The interactions between plants and their associated soil microbial community are therefore known to influence plant communities (van der Putten 1997, Kardol et al. 2007). This occurs through feedback mechanisms of plants influencing the composition of the soil community and soil organisms in turn affecting the performance of the plants (Bever et al. 1997). Alterations in the plant-associated soil community composition have been suggested to influence the performance and composition of plant communities by the moderation of available niches in a community (Bever et al. 1997). Therefore, if soil communities are plant-species specific, the diversity and density of plant species within communities could influence

plant–soil feedback effects (Levine et al. 2006). This implies that plant coexistence may be promoted by plant–soil feedbacks (Klironomos 2002, Petermann et al. 2008). A plant species' performance may be reduced (negative feedback) or may be increased (positive feedback) by feedbacks from the soil community. Such feedbacks can affect species competitive abilities within a community (Kardol et al., 2007).

Pathogens have been suggested to promote plant coexistence by regulating the relative abundance of dominant species or by reducing their competitive differences (Morris et al. 2007, Mordecai 2011). The Janzen-Connell effect proposes that negative feedbacks by conspecific seed predators and seedling herbivores are a factor in regulating species coexistence in tropical rain forests via density-dependent mortality (Janzen 1970, Connell 1971, Connell et al. 1984). Janzen-Connell type effects have been found in grasslands (Petermann et al. 2008) acting via negative plant–soil feedbacks (Bever 1994, Mills and Bever 1998, Bever 2003) suggesting negative plant–soil feedbacks could play a role in regulating plant communities (Bever 1994, Mills and Bever 1998, Bever 2003). Negative plant–soil feedbacks have consequently been suggested to contribute to the positive biodiversity effect on productivity in plant communities (Schnitzer et al. 2011, Kulmatiski et al. 2012) by regulation of potentially dominant species (Zuppinger-Dingley et al. 2011). Furthermore, plants not only accumulate pathogens and 'enemies' in their soil neighborhood but may also acquire beneficial effects from positive associations with soil biota (Sturz and Christie 2003, Avis et al. 2008, van der Heijden et al. 2008, van der Heijden and Horton 2009). Finally, since competition within a species is suggested to produce more negative plant–soil feedbacks than competition between species, plant individuals grown in species-rich communities are predicted to grow better (Kulmatiski et al. 2008) where species specific pathogens are diluted. The major challenge to unraveling how ecosystems function may

therefore be hidden in the network of plant–soil feedback dynamics (van der Heijden et al. 2008).

Combining Ability and Character Displacement

Combining ability was proposed as an evolutionary mechanism allowing for coexistence of species (Harper 1977, Aarssen 1983, 1985). Harper (1977) suggested that coexistence of species occurs through selection for niche differentiation thereby avoiding competitive interaction of species in a theory of ecological combining ability. For example, two species through ecological combining ability will be more productive in combination in comparison to their productivity in monocultures. The concept of competitive combining ability introduced by Aarssen (1983) suggests that species coexistence occurs by selection for balanced competitive abilities and is a consequence of the relative ability of two species to reduce the availability of a common resource to each other. Combining ability therefore implies that there is a co-evolution of competitors within a community.

The mechanism by which combining ability occurs may be through pre-adaption or the adaption of traits in response to selection to reduce resource competition through character displacement (Brown and Wilson 1956, Grant 1972, Taper and Case 1985, Dayan and Simberloff 2005) increasing functional distance between species within a community. Character displacement occurs therefore as the result of coevolution between competitors (MacArthur and Levins 1967, Connell 1971, 1980) and implies that species are more different from each other when they occur in a common community and in contrast more similar to each other in separate communities. Thus it would be expected that selection for reciprocal interactions occurs within mixed species communities (Schluter and Grant 1982). Selection, or local adaptation, for reduced competition between plant species and the consequential effects of their associated soil biota could conceivably be the key to understanding the

potential for increased resource uptake when plant species are combined in a diverse community.

Genetic, Epigenetic and Maternal Effects

Plants may adapt to their local biotic environment possibly even through genetic differentiation within plant species such that environmental selective pressures sort out and establish suitable genotypes from the gene pool (Stebbins 1969, Bossdorf et al. 2008a). It is therefore conceivable that a sorting out of pre-existing genotypes within the original sown seeds may have taken place over the eight years of the Jena experiment. Selection may have occurred for genotypes that perform better in mixtures and monocultures.

The local biotic environment can moreover lead to adaption of a particular plant species to that environment. In plants, local adaption to the diversity of the surrounding plant community and the associated soil biota can occur (Lipowsky et al. 2011). The mechanism by which this occurs could be via genetic, epigenetic or maternal effects. In the case of the latter two non-genetic mechanisms are responsible for adaptation (Roach and Wulff 1987, Rossiter 1996), which nevertheless may be heritable through carry-over for some generations (Russo et al. 1996, Whitelaw and Whitelaw 2006, Bird 2007, Jablonka and Raz 2009). Maternal effects occur when the environment of the mother has a direct influence on the phenotype of the offspring (Roach and Wulff 1987). Maternal effects can therefore influence not only the fitness of an individual in the absence of genetic change but also the frequency of a phenotype (Rasanen and Kruuk 2007). Phenotypic plasticity, defined as the ability of a genotype to express different phenotypes in different environments, may overlap with epigenetic and maternal effects (Bossdorf et al. 2008b).

Thesis Outlook

In this study I examined possible mechanisms for the observed positive temporal effect of plant species richness on biomass production in an on-going long-term biodiversity experiment established in Jena, Germany. It is conceivable that through plant–plant interactions, neighborhood diversity could lead to selection for plant types that are more productive in a similar environment through the selective difference between inter- and intraspecific competition; as assessed in monoculture and mixture treatments. We hypothesized therefore that there is selection for increased performance of mixture types in mixtures and of monoculture types in monocultures. To assess this, I collected plants of selected European grassland species from monoculture communities and mixed species communities from the Jena Experiment and reassembled them in new experimental monoculture vs. mixed-species test communities.

To examine whether selection occurs in plant communities for individuals that are more productive in either monoculture or mixed-species communities, I first used a diallel experimental design for the full combining of species within and among four plant functional groups each represented by three species. I wanted to address the question of whether over eight years in mixed grassland communities, individuals in mixtures are selected with high interspecific combining ability via increased division of labor indicated by greater trait divergence between species (Chapter 1).

Plant–soil feedbacks can influence plant community functioning and are proposed to increase complementarity among species (Eisenhauer 2012). In an effort to assess the positive influence of soil feedbacks on the complementarity of plants selected in experimental mixtures or monocultures of grassland plant species over eight years, I collected plant material and soil inocula from monocultures and mixed species plots and grew them in test communities in a common garden experiment. Additionally I asked whether selection occurs

in monocultures for plant individuals with increased defense against the pathogens commonly accumulating in monocultures (Chapter 2).

I explore the possibility that metabolic changes may occur in response to selection in a community with a selection history of either monoculture or mixtures over 8 years. Fourier transform infrared spectroscopy can produce a biochemical signature of a selected sample possibly indicating changes in biochemical composition, thereby determining metabolic alterations within species subjected to changes in environmental conditions (Chapter 3).

The results presented in this dissertation demonstrate exciting novel insights into the mechanisms governing plant species coexistence and evolution. Given the increased extinction risks of species in mixed grasslands, understanding the mechanisms whereby primary producers coexist and drive ecosystem productivity is critical in predicting anthropogenic impacts on mixed grassland systems. Primary producers are considered a major driver of carbon sequestration (West and Post 2002, De Deyn et al. 2008) therefore the loss of species within an ecosystem may reduce the system's capacity for atmospheric carbon removal (Hooper et al. 2012). Furthermore, as the expanding human population increases pressure on land use and food requirements, more efficient fodder production in agriculture will be imperative. The selection of plants that are highly productive in mixtures may therefore be essential to meet increased requirements on the same or even reduced acreage. Our study demonstrates that plant individuals selected in species-diverse communities replanted in similarly diverse communities have significantly higher performance than plant individuals selected in monocultures.

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Chapter One

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Selection for division of labor in mixed plant communities drives increasing biodiversity effects

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Division of labor promotes maximal resource use and niche space filling in bacterial[1] and animal[2] communities. In experimental plant communities, biodiversity-ecosystem function relationships have been found to strengthen over time[3,4], often attributed to increased complementarity[5] and plant-soil feedbacks[6]. However, the mechanisms underlying this trend have never been tested. We propose a novel mechanism: the selection of plant genotypes for division of labor via character displacement increasing resource-use complementarity. We tested this hypothesis by comparing performance and functional traits of plants after 8 years of selection in experimental monocultures or mixtures of grassland plant species when growing them in monocultures and mixtures. Mixture history communities showed higher net biodiversity effects and complementarity effects than monoculture history communities. When grown in mixed communities, functional trait differences between species in mixture history communities were larger than in monoculture history communities, indicating selection for trait divergence in mixtures. Our study demonstrates a novel mechanism for the increase in biodiversity effects over time, via selection for individuals adapted to their biotic environment. These results imply that there may be long-term impacts of species losses beyond the short-term decline in ecosystem functioning, as loss of capacity for division of labor reduces ecosystem functioning beyond the loss of species.

Division of labor across genotypes has been demonstrated within animal[2] and bacterial[1] populations, and even within clonal plants[7]. Such division of labor is related to the concept of combining ability in plant community ecology, and may promote species coexistence[8,9]. This mechanism may explain how in long-term grassland plant experiments, the effect of greater biodiversity in promoting stability and productivity increases over time[3,4]. This temporal effect has been suggested to arise from complementary resource use or dilution of species-specific pathogens. Complementary resource uptake, such as partitioning of soil resources[10],

is a primary mechanism for productivity to increase with species richness[3]. The parallel mechanism of pathogen-dilution by diversity, the Janzen-Connell effect[11,12], both promotes species coexistence via density-dependent mortality and limits productivity at low diversity due to high pressure of species-specific pathogens. Here we propose a novel mechanism: that increased biodiversity effects over time result from selection for increased division of labor between plant species in diverse plant communities. Character displacement[13], as reflected in functional trait differences, may drive such increasing complementarity between species[14]. Our hypothesis predicts larger functional trait differences between species in mixtures, resulting in increased complementarity and thus stronger positive biodiversity effects on productivity. Selection for increased division of labor could thus explain the experimentally observed[3,4] increasing biodiversity effects over time, and may have implications for the effect of biodiversity on agricultural and forestry production, where biodiversity may be essential in maintaining the pace of production gains[15].

According to our hypothesis, mixed-species communities composed of progeny of plants grown in high diversity should have greater complementarity than mixed-species communities derived from monocultures. We refer to such plants as mixture types, hypothesizing a heritable phenotype resulting from distinct genotypic or epigenetic features, including maternal effects, arising from selection pressures in diverse communities. Thus, our division of labor hypothesis predicts two endpoints of selection across biodiversity gradients, mixture types selected for high performance in mixtures and relatively poor performance in monoculture, and conversely monoculture types selected for high performance in monocultures and low performance in mixtures.

We tested our hypothesis by growing mixture and monoculture types of 12 plant species, collected from selection communities of monocultures, mixtures of 4-60 species from the same functional group and mixtures of species from four functional groups (grasses, small

herbs, tall herbs, legumes), in pots containing four individuals of one or two species in the glasshouse. Our selection communities (selection history) were experimental plots of an 8-year biodiversity field experiment in Jena, Germany[16], and test communities (planted diversity) were the pots containing four individuals each (Fig. 1). We assessed productivity in mixtures vs. monocultures to calculate biodiversity effects, partitioned into complementarity and selection effects[17]. We consider higher net biodiversity and complementarity effects for mixture types as evidence for division of labor, and also tested whether such division of labor was greater for multi-functional group mixture types than for mono-functional group mixture types. Furthermore, we tested for character displacement by measuring differences in height, specific leaf area (SLA) and reproductive biomass between species in mixtures.

Mixtures of mixture types had higher total community biomass than mixtures of monoculture types (Extended Data Table 1: $P = 0.024$), and these patterns were consistent across functional group combinations. Net biodiversity effects (NE) and complementarity effects (CE) were larger for mixture than for monoculture types (Fig. 2a, b, $P = 0.011$ for both, Table 1), indicating increased division of labor, with consistent results for a majority of functional group combinations (Fig. 2b). Furthermore, selection effects (SE) were higher for mixture types than monoculture types in most communities containing legumes (Fig. 2b). This might have been due to mixture types of non-legumes benefitting more from nitrogen fixed by legumes, and thus contributing more strongly to mixture performance.

The increases in biodiversity effects in mixtures of mixture types were mirrored by functional traits differences between species. Height differences between species were particularly large in mixtures of mixture type (Figure 3a; $P = 0.011$, Extended Data Table 2). Mixture types also showed greater specific leaf area (SLA) differences between species in mixtures than did monoculture types (Figure 3b; $P < 0.001$). Mixture test communities with legumes showed much larger SLA differences from monoculture to mixture types (Extended

Data Table 2, $P < 0.001$). Functional diversity (FD), calculated from height, SLA and reproductive biomass, showed the same pattern of greater functional trait divergence in communities of mixture types (Figure 3c; Extended Data Table 2).

We demonstrated increased mixture performance and biodiversity effects in test communities of mixture types, driven by increased division of labor due to character displacement of functional traits between species. Our results were obtained with 12 typical grassland species of four different functional groups, supporting our hypothesis that increased biodiversity effects can result from selection for increased division of labor and suggesting that these results may apply quite generally. Whereas mixtures may select for increased complementarity and character displacement between species, it is conceivable that selection pressures in monocultures select for greater defense against species-specific pathogens. However, this defense may come at the cost of reduced performance in mixture environments where species-specific pathogens are diluted. Because mixture and monoculture types experienced selection environments for only eight years, the standing variation at the beginning of the experiment may have already included genotypes pre-adapted for monoculture or mixture environments[18-20].

Niche differences between species decrease the strength of interspecific competition relative to intraspecific competition[21]. Thus, selection in high-diversity communities with high interspecific competition can be expected to favor genotypes with more distinct niches between species, reduced interspecific competition and thus increased division of labor. Reduced interspecific competition could also result from the extension of the total community niche[22] in addition to or instead of finer division of currently used resources. The notion of diversity as a driver of plant population differentiation has been suggested in theory[23] and a field study has demonstrated that differential selection for monoculture and mixture types in grassland species can occur[24], supporting the proposal that local evolutionary changes arise

from selection through competition[25]. In addition, species diversity has been shown to influence plant traits associated with light and resource uptake such as shoot, leaf and stem length[26,27]. In our study, mixture types across all functional groups showed greater differences in height and SLA between species in mixtures. These between-species differences may have resulted from directional selection or selection for increased plasticity in mixture types.

An additional potential mechanism underlying the performance of mixture and monoculture types in mixture and monoculture test communities is investment in defense against pathogens. In monocultures, species-specific pathogens can accumulate rapidly[8,28], thus selection would favor increased investment in defense rather than growth. Several lines of evidence support this idea. In a parallel experiment, we found positive plant−soil feedbacks for monoculture types and negative ones for mixture types, suggesting greater resistance or tolerance to species-specific pathogens in monoculture types (Extended Figure 2). Furthermore, in a reciprocal transplant experiment using some of the same populations as our study, four out of five species showed increased herbivore damage for mixture types[24]. Finally, evidence from infrared spectral fingerprints of monoculture and mixture type individuals from this study indicated significant differences in metabolic profiles between types for eight species (Extended Figure 3), potentially reflecting differential defense chemistry.

We demonstrated an interaction between selection community and test community diversity, with increased division of labor of mixture types. Selection for trait divergence between species in mixtures potentially explains the increased complementarity between mixture types in our study. These results offer a novel explanation for the emergence of stronger biodiversity effects over time in experiments. Capturing the potential of this production-enhancing division of labor in diverse communities may have profound impacts for agricultural and forestry applications. This novel mechanism also implies that species

losses can affect not only ecosystem functioning in the short term, but also the long-term trajectory of biological communities.

Acknowledgements

This study was supported by the Swiss National Science Foundation (grant number 130720 to B. Schmid). Thanks to Daniel Trujillo Villegas, Leigh Oesch, Theres Zwimpfer, Matthias Furler, René Husi, the gardeners of the Jena Experiment and student helpers for technical assistance. G.B. De Deyn acknowledges the NWO-ALW VIDI grant scheme for financial support.

Co-authors Contribution

B.S. and J.S.P. conceptualized the study; D.Z-D. designed the experimental procedure and carried out the experiment with the help of D.F. and staff of the Schmid-lab; B.S., D.Z-D. and D.F. analyzed the data; D.Z-D., B.S. and D.F. wrote the paper with input from J S.P., V.Y., and G.B.D.D. All authors, D.Z-D, B.S., J.P., V.Y., G.B.D.D., D.F., discussed design, field and glasshouse work and analysis.

Methods Summary

Cuttings of 12 perennial grassland plant species were taken from monocultures and mixtures of species of a single or four different plant functional groups (grasses, legumes, short herbs and tall herbs) established in plots of a field-based biodiversity experiment in Jena, Germany (http://www.the-jena-experiment.de). The cuttings were made in spring 2010, eight years after sowing of the experimental plots[16]. The species were *Festuca pratensis*, *Festuca rubra*, *Poa pratensis*, *Lathyrus pratensis*, *Onobrychis viciifolia*, *Trifolium repens*, *Plantago lanceolata*, *Prunella vulgaris*, *Veronica chamaedrys*, *Crepis biennis*, *Galium mollugo*, and *Geranium pratense*. Cuttings were transported to Zurich, Switzerland and planted in an experimental garden for propagation of cuttings or seed production, with caging to ensure pollination was only among cuttings from the same original experimental plot.

We made cuttings and collected seeds from the initial cuttings, raising cuttings and seedlings in pots in a glasshouse. We transplanted cuttings in monocultures of four plants or in two-species mixtures of two individuals per species into pots according to a diallel design for full combining of species within and among functional groups of available plant material (42 two-species combinations, Extended Data Figure 1). We transplanted seedlings into pots following the same procedure and design. All test communities were established with plants of three types of selection history: monoculture types and mixture types taken from mixtures of a single functional group (mono-functional group mixture types) and from mixtures of four different plant functional groups (multi-functional group mixture types). Test communities never contained plants of different selection history. Plant traits and biomass were measured 20 weeks after planting for the block established with cuttings and the block established with seedlings.

We used mixed models with cuttings vs. seedlings, test community diversity (monoculture, mono-functional group mixture and multi-functional group mixture), selection

history (monoculture, mono-functional group mixture and multi-functional group mixture type), legume presence in test community and functional group combination of test community as fixed effects. Glasshouse table, species combination of test community and pot within table were used as random effects. Interactions of fixed effects were used as further fixed terms and interactions between fixed and random effects as further random terms in the analyses. Biodiversity net (NE), complementarity (CE) and selection effects (SE) were calculated with additive partitioning according to Loreau & Hector[29], and functional diversity was calculated as Petchey & Gaston's FD[30].

Methods

Experimental setup

To test whether plant types selected for over eight years in mixtures outperform those types selected for in monocultures when assembled in mixture test communities, and vice versa for types selected for in monocultures, we first selected 12 of the 60 species in the Jena Experiment, Germany (50°55'N, 11°35'E, 130 m a.s.l, www.the-jena-experiment.de, see Roscher et al. 2004 for experimental details). Three species from each of the four functional grassland groups were selected: grasses (*Festuca pratensis, Festuca rubra, Poa pratensis*), the small herbs (*Plantago lanceolata*, *Prunella vulgaris*, *Veronica chamaedrys*); the tall herbs (*Crepis biennis, Galium mollugo, Geranium pratense*); and legumes (*Lathyrus pratensis*, *Onobrychis viciifolia*, *Trifolium repens*) (Extended Data Figure 1). These species have an eight-year community history growing in monocultures or mixtures consisting of plants belonging to a single functional group or to all four functional groups. In April 2010, we collected 4900 plant cuttings from 48 of the 84 plots in the Jena Experiment.

We used the majority of these plant cuttings to establish plots in an experimental garden in slug exclosure compartments at the University of Zurich, Switzerland (47°33′N, °37′E, Altitude 534 m) with an identical plant composition to the plots in Jena from which the cuttings were collected. We added a layer of potting soil (BF 4, De Baat; Holland; Extended Data Table 3) to the soil in each plot to make sure the plants established. Netting around each plot minimized the possibility of cross-pollination between the same species from different community histories. The cuttings were used for the propagation of further cuttings or to produce seeds for our study.

Using 25 of the plant cuttings of each species from the three field community histories, we generated further cuttings, from April 2010 until September 2010, in pots in the experimental glasshouse to set up the first block of the experiment. Thus, the cuttings used in

the experiment were the result of six rounds of propagation. Each set of cuttings was timed according to the slowest growing species, every three to four weeks.

During the summer of 2010, we collected seed material from the experimental garden plots for the second block of this experiment. The seed material was dried in a glasshouse compartment. We cleaned the seeds from the husks/pods and stored them at 10-15°C, 50 % humidity in a climate chamber. Once all the seeds were cleaned, they were treated to cold stratification at 5°C for two months. Seeds were germinated in a 10.5-h day regime with 14–19 °C day- and 10–16 °C night temperature.

In November 2010 we transplanted randomly-selected individuals which were cuttings from the 25 original cuttings. We made monocultures of four plants or 2-species mixtures of two + two plants into pots (4275 cm^3) filled with neutral agricultural soil (Extended Data Table 3) according to a diallel design containing all possible combinations of species within and among functional groups according to available plant material (42 types of 2-species combinations, Extended Data Figure 1). We replicated each of the plant compositions three times. Cuttings of the legume *Onobrychis viciifolia* were not successfully propagated and were therefore excluded from the first block. Thus, of the 139 planned treatment combinations, 129 species combinations were realized. In October 2011 we transplanted seedlings into pots following the same procedure and design. 120 treatment combinations could be realized. All test communities of the diallel design were established with plants of three types of selection history: monoculture types taken from monocultures and mixture types taken from mixtures with species of a single (mono-functional group mixture types) or four different plant functional groups (multi-functional group mixture types). Test communities never contained plants of different selection history. Plant traits and biomass were measured 20 weeks after planting for the block established with cuttings and the block established with seedlings. There were 1048 pots, with 4192 individuals in total across both

blocks. To remove effects of plant-soil feedbacks, we used a neutral growth substrate (50% agricultural soil, 25% sand, 25% Perlite; Ricoter AG, Aarberg; Extended Data Table 3) throughout the experiment. Once the plants were transplanted into the pots, glasshouse conditions were set to natural summer day length and day temperatures of 20°C and night-temperatures 17°C. To supplement sunlight, additional light was provided at a maximum of 30 kLux (Metallhalogenlamps 400 Watt, Iwasaki MT 400 DL/BH). Shading was at 20 kLux. To compensate for overheating, an adiabatic cooling system (Airwatech; Bern, Switzerland) was used. The plants were watered in the trays to make sure that each individual received equal water volume. Seedlings that died in the first 2 weeks were replaced with seedlings of the same age.

Measurements and harvest

Height and leaf number were measured at planting to ensure that the cuttings and seedlings were standardized. Height was measured again at eight weeks after transplanting into the pots. The aboveground biomass of each individual was harvested at ground level 20 weeks after transplanting into the pots. The inflorescence, if present at harvest, was collected separately and the dry biomass weighed as an indication of reproductive effort. Specific leaf area (SLA) was measured at harvest.

Statistical Analysis

We tested performance of plants in monoculture vs. mixture test communities selected for in either monoculture or mixed species communities of 8 years using the mean aboveground dry biomass of populations within our experimental communities as our response variable. We used general mixed models using residual maximum likelihood (REML) and results summarized in analyses of variance (ANOVA) tables. Significance tests were based on approximate F-tests using appropriate error terms and denominator degrees of freedom. The fixed terms in the models were: block (cuttings vs. seedlings), planted diversity (monoculture

vs. mixture), planted functional group diversity (monoculture vs. mono-functional group mixture vs. mixed functional group mixture), origin history (monoculture selection vs. mixture selection), functional group diversity of the origin history (monoculture selection vs. mono-functional group selection vs. mixed-functional group selection), presence or absence of legumes in the test community, functional group combination within a test community and interactions among these. Table, pot within table, and species combination were used as random terms. The full model is presented in Extended Data Table 1.

To assess biodiversity effects we followed the additive partitioning method of Loreau and Hector[29], net effect (NE) partitioned into complementarity effect (CE) and selection effect (SE). Calculations were based on the difference between the observed yield of each species in the mixture and mean monoculture yield for that species in the corresponding block and for that specific origin history. A concise version of the biodiversity effects analysis is presented in Table 1 in the main text.

Absolute differences in height and SLA, as well as functional diversity (FD), were calculated to test the mechanism underlying the differences in performance measured by biomass. FD was calculated using Petchey and Gaston's FD[30] using mean values (calculated by species, origin history, planted diversity, block) of height, SLA and reproductive biomass as our functional traits. The statistical model was based on the final model in the main analysis. Statistical analyses were conducted using the software products R, version 2.15.3 (R Development Core Team, 2013), and GenStat, version 16 (VSN International Ldt. 2013).

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Table 1: Results of simplified mixed-effects ANOVA for Net Effect (NE, untransformed), Complementarity Effect (CE, square-root transformed), Selection Effect (SE, square-root transformed), (numDF: degrees of freedom of term, denDF: degrees of freedom of error term [which can be fractional in REML analysis], F

statistic, *P*).

		NE			CE			SE		
Source of variation	numDf	denDf	F	*P*	denDf	F	*P*	denDf	F	*P*
Selection history (SH):										
Monoculture vs. Mixture (SH mo. vs. mi.)	1	62.9	6.04	**0.017**	64.4	8.49	**0.005**	66.7	0.01	0.921
Functional group combination (FG comb.)	9	61.4	2.4	**0.021**	61.1	1.05	0.410	64.7	1.91	0.067
SH mo. vs. mi x FG comb.	9	66.2	1.43	0.193	69.0	1.32	0.242	70.5	0.90	0.527
Random terms	n	VC	s.e.		VC	s.e.		VC	s.e.	
SH mo. vs. mi x Species combination	86	0.942	0.415		0.123	0.086		0.117	0.056	
Residual	545	8.314	0.547		2.254	0.148		1.245	0.082	

Figure 1. Plant material from experimental field monoculture vs. mixed species selection communities established in Jena, Germany in 2002; were collected in 2010 and assembled in new experimental glasshouse monoculture vs. mixed species communities in 2010/11. We expected mixed species communities would have higher productivity if assembled from plants collected from mixed species plots in Jena and vice versa for monocultures. Different shades of green represent the hypothesized selection for monoculture or mixture types between 2002 and 2010, respectively.

Figure 2. a) Biodiversity effects were assessed by additive partitioning to net effect (NE) partitioned into complementarity effect (CE) and selection effect (SE). b) The plots are ordered by functional group combinations: grasses (g), small herbs (sh), tall herbs (th), legumes (l).

Figure 3. Differences for key plant traits, a) height, b) specific leaf area (SLA) as measured by trait values between species for each selection history. c) Functional diversity calculated from height, SLA and reproductive biomass for species in each selection history.

Fig. 1

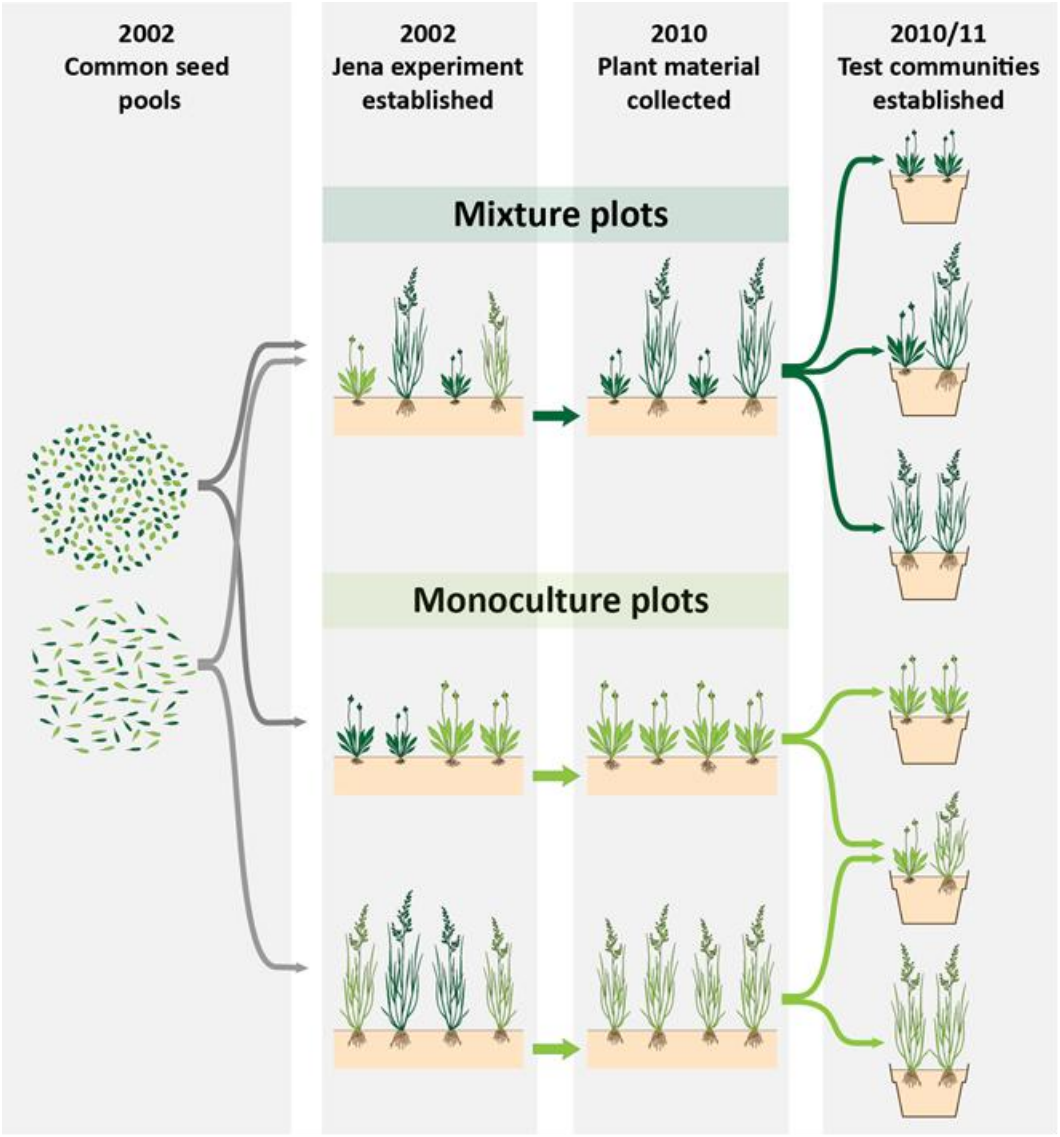

2002
Common seed
pools
2002
Jena experiment
established
2010
Plant material
collected
2010/11
Test communities
established
Mixture plots
Monoculture plots

Fig. 2

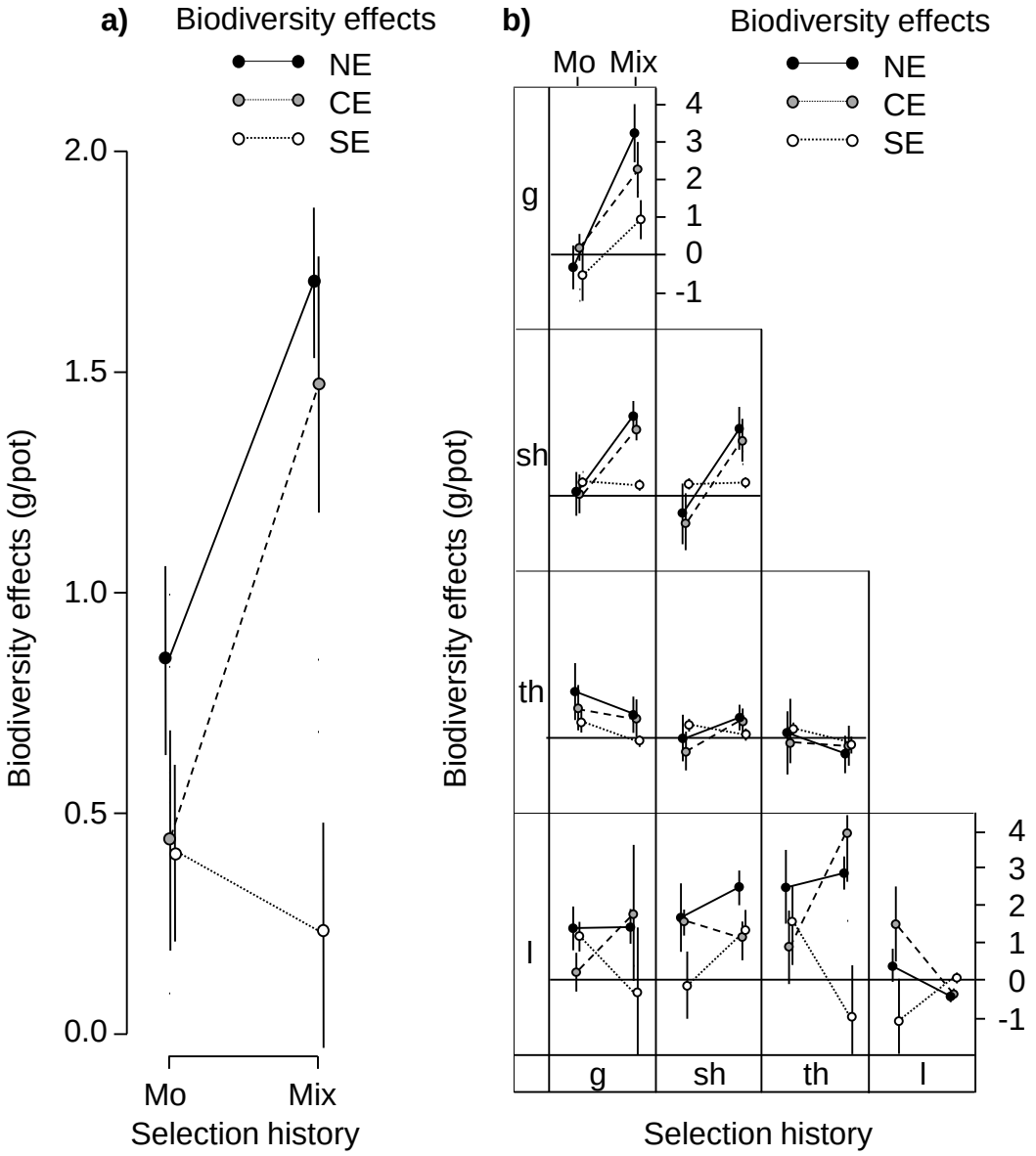
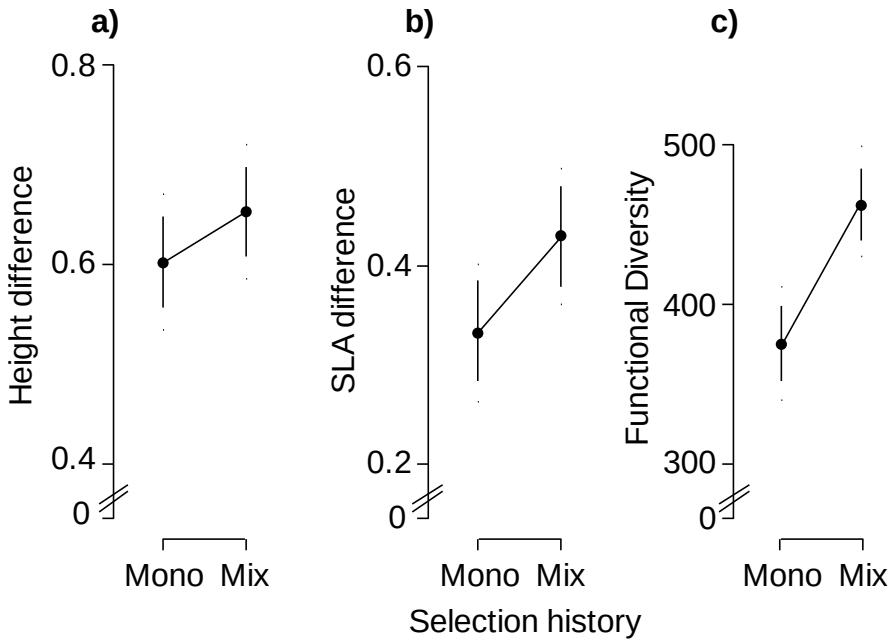

a)
Biodiversity effects
NE
CE
SE
Biodiversity effects (g/pot)
2.0
1.5
1.0
0.5
0.0
Mo
Mix
Selection history
b)
Biodiversity effects
NE
CE
SE
Mo
Mix
g
sh
th
l
4
3
2
1
0
-1
Biodiversity effects (g/pot)
Selection history

Fig. 3


a)
b)
c)
Height difference
SLA difference
Functional Diversity
0.8
0.6
0.4
0
0.6
0.4
0.2
0
500
400
300
0
Mono Mix
Mono Mix
Mono Mix
Selection history

Extended Data

Extended Data Table 1 | Results of mixed-effects ANOVA for the community dry aboveground biomass 20 weeks after transplanting plants into pots (numDF: degrees of freedom of term, denDF: degrees of freedom of error term [which can be fractional in REML analysis], F statistic, *P*).

Source of variation	numDf	denDf	F	*P*
Seedlings vs. cuttings (Block)	1	65.2	5.89	**0.018**
Selection history (SH; next two contrasts):				
Monoculture vs. mixture (SH mo. vs. mi.)	1	717.4	0.55	0.457
Mono-functional group vs. multi-functional group mixture (SH mo.fu. vs. mi.fu.)	1	707.1	0.11	0.735
Planted diversity (PD; next two contrasts):				
Monoculture vs. mixture (PD mo. vs. mi.)	1	41.2	4.84	**0.034**
Mono-functional group vs. multi-functional group mixture (PD mo.fu. vs. mi.fu.)	1	41.9	0.09	0.767
SH × PD (next two contrasts):				
SH mo. vs. mi. × PD mo. vs. mi.	1	714.3	5.15	**0.024**
SH mo.fu. vs. mi.fu. × PD mo.fu. vs. mi.fu.	3	713.4	1.30	0.274
Legumes (Leg.)	1	44.5	38.02	**<0.001**
Functional group combination (FG comb.)	7	43.8	2.51	**0.029**
SH mo. vs. mi. × Leg.	1	727.7	4.04	**0.045**
PD mo. vs. mi. × Leg.	1	46.3	0.14	0.712
SH mo. vs. mi. × FG comb.	7	729.2	1.17	0.318
PD mo. vs. mi. × FG comb.	2	42.4	0.41	0.664
SH mo. vs. mi. × PD mo. vs. mi. × Leg.	1	704.0	1.27	0.260
SH mo. vs. mi. × PD mo. vs. mi. × FG comb.	2	731.1	1.58	0.207
SH mo.fu. vs. mi.fu. × PD mo.fu. vs. mi.fu. × Leg.	3	725.8	0.45	0.719
SH mo.fu. vs. mi.fu. × PD mo.fu. vs. mi.fu. × FG comb.	8	719.6	2.23	**0.023**
Block × SH × PD (next four contrasts):				
Block × SH mo. vs. mi.	1	729.9	2.9	0.089
Block × PD mo. vs. mi.	1	50.5	0.00	0.986
Block × SH mo. vs. mi. × PD mo. vs. mi.	1	722.1	2.08	0.149
Block × SH mo.fu. vs. mi.fu. × PD mo.fu. vs. mi.fu.	5	361.9	0.68	0.642
Random terms	n	VC	s.e.	
Block × Glasshouse table	44	0.662	0.235	
Species combination	62	0.772	0.764	
Block × Species combination	109	2.905	0.822	
Residual	855	6.708	0.367	

Extended Data Table 2 | Results of mixed-effects ANOVA for height difference, SLA difference and functional diversity (numDF: degrees of freedom of term, denDF: degrees of freedom of error term [which can be fractional in REML analysis], F statistic, *P*).

	Height difference				SLA difference				FD			
Source of variation	numDf	denDf	F	*P*	numDf	denDf	F	*P*	numDf	denDf	F	*P*
Seedlings vs. cuttings (Block)	1	36.1	0.10	0.757	1	28.6	26.21	**<0.001**	1	33.7	3.07	0.089
Selection history (SH)												
Monoculture vs. Mixture (SH mo. vs. mi.)	1	30.7	7.25	**0.011**	1	27.2	20.62	**<0.001**	1	32.3	17.42	**<0.001**
Planted diversity (PD)												
Mono-functional group vs. multi-functional group mixture (PD mo.fu. vs. mi.fu.)	-	-	-	-	1	38.0	1.24	0.272	1	37.8	4.23	**0.047**
Functional group combinations with legumes (Leg.)	1	37.1	8.20	**0.007**	1	38.7	0.28	0.602	1	39.2	3.69	**0.062**
Functional group combinations (FG rest)	8	36.8	0.90	0.525	7	38.5	3.25	**0.008**	7	38.2	4.64	**<0.001**
Block x SH mo. vs. mi.	1	83.4	37.24	**<0.001**	1	82.3	91.76	**<0.001**	1	85.3	36.41	**<0.001**
Block x PD mo.fu. vs. mi.fu.	-	-	-	-	1	27.7	4.34	**0.047**	1	33.3	2.84	0.101
Block x Leg.	1	36.4	0.43	0.517	-	-	-	-	1	34.6	5.97	**0.02**
Block x FG rest	8	36.1	0.55	0.809	-	-	-	-	-	-	-	-
Block x FD all	-	-	-	-	8	29.2	1.20	0.334	-	-	-	-
SH mo. vs. mi. x PD mo.fu. vs. mi.fu.	-	-	-	-	-	-	-	-	1	32.3	1.37	**0.251**
SH mo. vs. mi x Leg.	1	31.1	2.11	0.156	1	27.6	18.13	**<0.001**	1	32.7	13.00	**0.001**
SH mo. vs. mi. x FG rest	8	30.7	2.48	**0.034**	8	27.2	0.60	0.773	-	-	-	-
Block x SH mo. vs. mi. x PD mo.fu. vs. mi.fu.	-	-	-	-	-	-	-	-	1	88	12.08	**<0.001**
Block x SH mo. vs. mi. x FG all	-	-	-	-	9	83.7	8.96	**<0.001**	-	-	-	-
Block x SH mo. vs. mi. x Leg.	1	84.9	20.11	**<0.001**	-	-	-	-	1	85.8	5.41	**0.022**
Block x SH mo. vs. mi. x FG rest	8	84.6	3.22	**0.003**	-	-	-	-	21	92.3	2.51	**0.001**
Random terms	n	VC	s.e.		n	VC	s.e.		n	VC	s.e.	
Species combination	50	0.006	0.031		50	0.040	0.015		50	4139	4449	
SH mo. vs. mi x Species combination	93	0.005	0.002		89	0.007	0.003		93	6784	2010	
Block × Species combination	88	0.160	0.041		85	0.025	0.008		88	14483	4023	
Residual	219	0.002	0.003		208	0.005	0.001		219	2152	350	

Extended Data Table 3 | Composition of 1 g of substrate GVZ Tref GO PP 7000 (BF4: black peat; white peat; clay; mineral fertilizer, 1.3 kg/m3; GVZ; De Baat, Holland) and neutral agricultural soil (50% sugarbeet soil, sieved; 25% washed river sand, 0–2 mm; 25% perlite, 2–6 mm; AGR; RicoterAG, Aarberg) using elemental analysis ($^{+}$) and as determined by lbu, program 40 analysis, Laboratory for Soil Analysis, Thun, Switzerland (*). All units in mg/L are per liter extract solution.

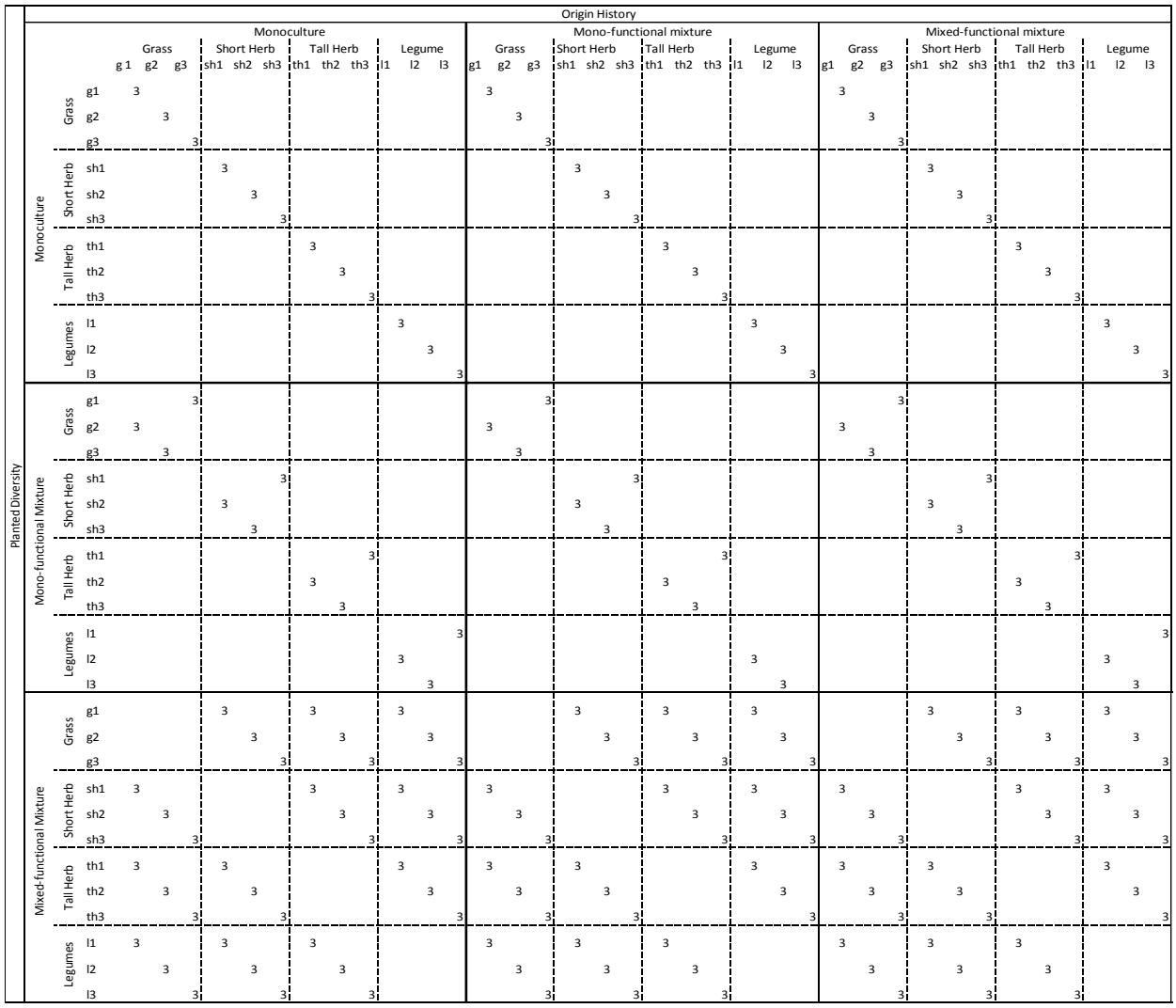
Parameter	Unit	GVZ	AGR
Organic matter*	%(mass)	55	3.5
Clay*	%(mass)	1.0	1.0
Silt*	%(mass)	1.0	1.0
Carbon^{+}	µg/g	411.5	34.60
Hydrogen^{+}	µg/g	47.7	4.6
Nitrogen^{+}	µg/g	8.0	2.1
pH*		5.4	7.9
Nitrate†	mg/L	439	730
Ammonium†	mg/L	0.7	2.5
Phosphorus†	mg/L	20	0.3
Potassium†	mg/L	54	127
Calcium†	mg/L	119	187
Magnesium†	mg/L	43	40

Extended Data Figure 1 | Combinations of species from three different selection origin histories; monoculture = plot containing one species, mono-functional group= plot containing at least four species of the same functional group of plants, mixed-functional group = plot containing at least four species of four different functional groups, were regrown in monocultures and either mono- or mixed-functional group experimental communities (Planted diversity). Twelve species in the Jena Experiment were chosen from all four functional groups: g = grass (*Festuca pratensis, Festuca rubra, Poa pratensis*), sh = small herb (*Plantago lanceolata, Prunella vulgaris, Veronica chamaedrys*), th = tall herb, (*Crepis biennis, Galium mollugo, Geranium pratense*), l = legume (*Lathyrus pratensis, Onobrychis viciifolia, Trifolium repens*).

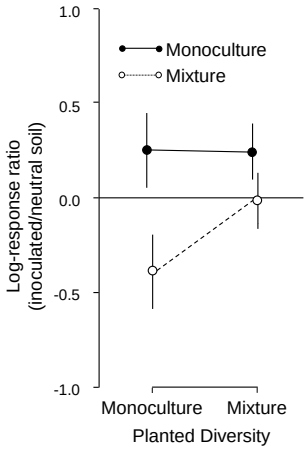
Extended Data Figure 2 | Values for soil feedbacks (log-ratio of values in inoculated vs. neutral soil) calculated from mean log-transformed aboveground biomasses of plants selected for in Jena monoculture and mixed species communities for 8 years grown in new experimental monocultures or mixtures.

Extended Data Figure 3 | Ordinations (NMDS) of second derivative of spectral wavenumbers of target species, showing clear effects of selection history on plant individuals from Jena monoculture and mixed species communities for 8 years. This can be an indication of different defense chemistry.

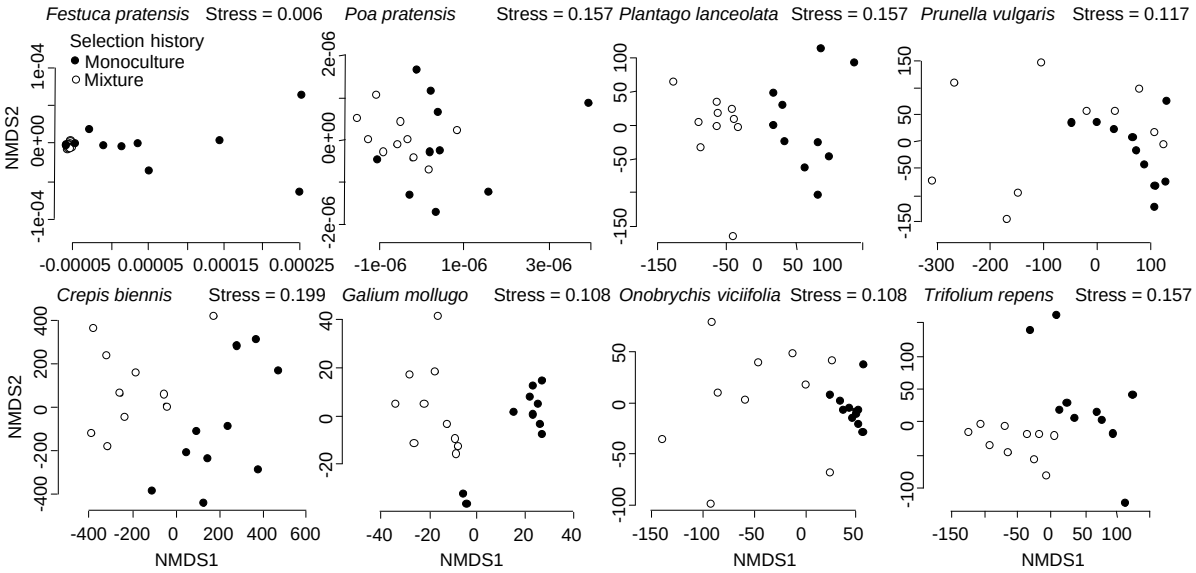
Extended Data Figure 1



Extended Data Figure 2



Extended Data Figure 3



Chapter Two

(Formatted for submission to Ecology)

Plant- and soil-legacies drive biodiversity effects in biodiversity experiments

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Abstract

Plant–plant and plant–soil interactions can help maintain plant diversity and ecosystem functions. Changes in such interactions may underlie experimentally observed increases over time in biodiversity effects, yet little is known about such legacy effects. Plant-legacy effects may occur if selection of low- and high-diversity genotypes pre-adapted to low- or high plant diversity conditions occurs during the course of biodiversity experiments. Soil-legacy effects that promote plant biodiversity effects may occur if plant growth in monocultures is increasingly limited by species-specific pathogen accumulation over time. We tested how these two types of legacy effects modify biodiversity effects on productivity in mesocosms with one or four species and whether such effects increase the resistance of the communities to invasion by weeds. The plant legacies were tested by using progeny of plants selected for 8 years in monoculture or mixture plots of a large biodiversity experiment and grown on neutral soil or soil inoculated with the 8-year old soil from the same experiment to test soil legacy effects, and the interaction of these effects. For each of the four factorial combinations of plant- by soil-legacy treatments we calculated biodiversity effects partitioned into complementarity and selection effects. Plant and soil legacy interactively modified the effects on biodiversity on plant biomass production and resistance to weed invasion. In particular, complementarity effects were largest for mesocosms assembled from progeny of plants selected in mixture and grown on soil with inoculum from mixture plots. This suggests that an evolutionary increase in biodiversity effects on productivity in experimental grassland communities is possible but may depend on the presence of a co-evolved community of soil organisms.

Key Words: biodiversity, selection, complementarity, plant–soil feedbacks, weed suppression, ecosystem function.

Introduction

Diminishing species richness reduces ecosystem functioning (Balvanera et al. 2006, Isbell et al. 2011, Cardinale et al. 2012), including most prominently primary productivity. Long-term experimental manipulations of plant biodiversity have shown that over time, higher plant species diversity increases stability and productivity within a community with an increasing effect over time (Tilman et al. 2006, Reich et al. 2012). The mechanisms by which this temporal effect occurs are only beginning to be considered, but will likely relate to mechanisms supporting coexistence in diverse communities over time. Often such plant coexistence is interpreted as a potential consequence of niche differences among species (Savage 1958, Silvertown 2004). There is evidence indicating that different plant species separate along environmental niche axes (Silvertown 2004), for example by partitioning soil nutrients (Roscher et al. 2008). As a consequence of partitioning, a larger number of species can extract a greater amount of resources from the environment, providing a general hypothesis for positive biodiversity–productivity relationships and other positive biodiversity effects via resource-use complementarity among species (Loreau and Hector 2001, Tilman et al. 2001, HilleRisLambers et al. 2004). This general hypothesis can explain a positive biodiversity effect, but it does not explain why the effect increases over time.

One possibility is that evolutionary changes over the course of time can lead to increased resource-use complementarity among species in mixture (Zuppinger-Dingley et al, in prep.). The local biotic environment can lead to adaption of a plant species to that environment. For example, for five plant species in the Jena Experiment in Germany, greater performance was observed for seedlings planted in diverse mixtures. In some cases this greater performance was observed only when those seedlings were grown from seeds collected from such diverse mixtures (Lipowsky et al. 2011). This may possibly be due to genetic differentiation within population simply by differential survival, with selective pressures sorting out suitable genotypes (Stebbins 1969, Bossdorf et al. 2008). It is therefore

conceivable that a sorting out of pre-existing genotypes within the original sown seeds may have taken place over the eight years of the Jena Experiment. Selection may have occurred for genotypes that perform better in mixtures and monocultures. We refer to the history of growth in monocultures or mixtures as plant legacy.

In addition to such plant legacy, it is increasingly recognized that soil legacy may play a role in species coexistence in mixed plant communities. Recently plant–soil feedbacks have been suggested as a source of complementarity between plants (Eisenhauer 2012, Kulmatiski et al. 2012). The mechanisms driving these feedbacks may be analogous to the Janzen-Connell effect of aboveground herbivores (Janzen 1970, Connell 1971). Such mechanisms can be defined in a broad sense to include negative plant–soil feedbacks (Bever 1994, Mills and Bever 1998) and can structure grassland plant communities (Petermann et al. 2008, van der Heijden et al. 2008). Pathogens are suggested to promote coexistence by regulating relative abundance or by reducing the differences in fitness between species (Mordecai 2011). Negative plant–soil feedbacks could therefore regulate mixed plant communities (Bever 1994, Mills and Bever 1998, Zuppinger-Dingley et al. 2011) and thus contribute to the positive effect of biodiversity on productivity in plant communities (Schnitzer et al. 2011, Kulmatiski et al. 2012). Furthermore as a consequence of reduced rate of build-up of species-specific pathogen pressure, plants in species-rich communities will be more productive than plants in monocultures.

In addition, communities with higher species diversity are considered to be more resistant to invasion by weeds as species rich communities have less available niche space for weeds to establish (Levine 2000, MacDougall et al. 2009). Biodiversity may therefore play a key role in the resistance of a community to weed invasion (Kennedy et al. 2002, Turnbull et al. 2010). Indirectly weeds may benefit from either pathogen release or from symbiotic mutualists (Reinhart and Callaway 2006) may contribute to the susceptibility of plant

communities to invasion by weeds (Klironomos 2002). The above- and belowground consequences of plant diversity for weed invaders lead to contrasting predictions. If aboveground effects dominate, where the increased biodiversity effects lead to greater complementarity and thus less available niche space, susceptibility to weeds will be reduced. If belowground effects dominate, with pathogen dilution and more numerous soil mutualists, susceptibility to weeds will be increased.

In the present study we investigated the interaction between plant- and soil-legacy effects and their interaction on biodiversity effects. Biodiversity effects are partitioned into "complementary effects", reflecting performance of mixtures beyond the sum of their parts, and "selection effects" , where performance in mixtures is being determined by performance in monoculture (Loreau and Hector 2001). Plant-legacy effects were tested by assembling mesocosms of one or four species from progeny of plants selected for 8 years in monoculture and mixture plots of a large biodiversity experiment. Soil-legacy effects were tested by inoculating the soil in half of the pots with 8-year old soil from the same biodiversity experiment from both monoculture and four functional group plots.

We hypothesized that complementarity effects will be larger in mixed plant communities constructed with mixture legacy in contrast to such communities constructed with monoculture legacy progeny.

and 2 inoculated with soil from mixtures. Furthermore, 3 we hypothesized that as a consequence these communities could also be more resistant towards invasion by weeds.

Methods

We used the Jena Experiment, a large biodiversity experiment in Germany (50°55'N, 11°35'E, 130 m a.s.l.; Table 1) as our source of plant and soil material reflecting 8 years of legacy effects. This plant and soil material was then transferred to a new experiment with

monocultures and 4-species mixtures in mesocosms in an experimental garden in Zurich, Switzerland (47°33′N, 8°37′E, 534 m a.s.l.). We chose eight out of the 60 grassland plant species occurring in the Jena Experiment, Germany. Two species were chosen from each of four plant functional groups: grasses, legumes, tall herbs and small herbs. In April 2010 we collected 4900 plant cuttings from monocultures and mixtures in Jena.

These plant cuttings were used to establish plots in slug-exclosure cages in the experimental garden in Zurich in an identical plant composition to the 48 plots in Jena from which the cuttings had been collected. We added a layer of potting soil (BF 4, De Baat; Holland) to each plot, filled with field soil without plant growth for 10 years, to ensure that each cutting established well in the new plot. The plots were caged individually with white mesh fabric to minimize potential cross-pollination between plots. During the summer of 2010 we collected fruits from the cutting-derived plants. We removed the seeds from the fruits and stored them at 10–15 ºC and 50 % humidity in a climate chamber. The seeds were cold stratified by storing them in a cold room (5 ºC) for four months to simulate a winter period before germinating. Seeds were germinated in a 10.5-h light per day regime with 14–19 °C during the light and 10–16 °C during the dark period.

The seedlings were used to assemble test communities of 16 individuals per mesocosm of 25 × 25 cm area and 25.5 cm depth. Monoculture test communities contained individuals of a single species, whereas mixture test communities contained four times four individuals of different functional groups; that is, all mixtures were 4-species, 4-functional groups mixtures. There were eight monoculture species and 16 unique mixture species compositions (Appendix A, Table 1). Each species occurred in the same number of mixture species compositions, namely 8 different ones. Monoculture and mixture test communities were derived from monoculture and mixture selection communities, yielding four combinations of planted-diversity × plant-legacy treatments. Plant-legacy corresponds to monoculture vs. mixture Jena growth history. For each of the eight monocultures and 16 mixture combinations in each of

the two selection histories we planted six replicate pots in early May 2011, of which three replicates were planted in soil with inoculum serving as soil legacy and three in soil with a neutral soil inoculum. *Poa pratensis* with monoculture plant legacy did not germinate well , so monocultures were reduced to four individuals in pots of 11 × 11 cm and 12 cm depth) and two of the planned mixture combinations (*Poa pratensis, Plantago lanceolata*, *Trifolium repens*, *Crepis biennis* and *Poa pratensis, Prunella vulgaris, Trifolium repens*, *Crepis biennis*) with this species were excluded and one combination (*Poa pratensis, Prunella vulgaris, Galium mollugo, Onobrychis viciifolia*) was replicated in two instead of the planned three replicates.

For each of the above treatment combinations three pots were used for each of the two soil-legacy treatments: neutral soil vs. neutral soil with inoculum from the Jena Experiment. The neutral soil consisted of 50 % agricultural soil, 25 % sand and 25 % Perlit (Ricoter AG, Aarberg). The neutral soil was inoculated with soil-inoculum at 4 % of the total mass using the soil from either monocultures or mixtures in the Jena Experiment. To create the Jena soil inoculum to serve as soil legacy test soil bulked soil samples (0–5 cm depth) were taken from the respective plots from which the plant material had been collected. Large objects, such as stones, were removed and the collected soil samples from each plot were homogenized using a 5 mm sieve. The soil inoculum for the monoculture pots were collected from each corresponding original monoculture plot in the Jena Experiment such that each species was inoculated with soil from that specific species monoculture soil. For monoculture legacy plants soil from the monoculture plots was “home” soil whereas for mixture legacy plants such inoculum was “away” soil and vice versa. Soil inoculum for the mixture pots were pooled samples collected from all the 4-functional group plots from which plant material was collected. For better comparison with inoculated soil, the neutral soil received sterilized inoculum from the respective Jena plots at 4 % of the total mass.

Each of the replicate pots was placed in three blocks in the experimental garden in Zurich. Each block contained one pot per treatment combination (planted species identity/species composition × plant legacy × soil legacy) and was surrounded by a slug-exclosure fence. The treatment combinations were completely randomized within blocks. Weeding was carried out throughout the growing season at fortnightly intervals. The weed material was kept, dried and its aboveground biomass determined. Weeds were defined as those species that had not been included among the planted species in the corresponding pot. Seedlings of planted species that did not survive during the first two weeks were replaced with spare seedlings of the same species, plant legacy and age.

Three weeks after planting all plants were cut back to 5 cm to standardize their sizes. Fifteen weeks after transplanting all plants were harvested at 3 cm above ground to determine the biomass increase within the 12-week period late May–mid-August 2011. The survival of plants within each community was noted after 3 and 15 weeks. A planned second-year harvest could not be carried out because a large proportion of species did not survive after the winter 2011/12 with an unusually cold period in February 2012.

Data analyses

We analyzed the log-transformed dry aboveground biomass of planted species and the proportion of weeds of the total dry aboveground biomass (planted species + weeds) as a function of the above-mentioned treatments using mixed-model analysis of variance. The fixed terms in the analysis were planted diversity (monoculture vs. mixture test communities), plant legacy (monoculture vs. mixture selection communities), soil treatment (neutral vs. soil legacy) and interactions among these. Block and species composition were used as random terms. The smaller monocultures of *Poa pratensis* with monoculture plant legacy were excluded from the above analysis.

To calculate biodiversity effects for mixture test communities we used the additive partitioning method of Loreau & Hector (2001), which partitions net biodiversity effects into complementarity and selection effects. The net biodiversity effect is the difference between a mixture and the average of the monocultures of the species making up the mixture. If the different species contribute similarly to mixture biomass, is reflected in a large complementarity effect. If a few or a single species dominate the mixture biomass, net effect is reflected in a large selection effect (note that in this study this term refers to the calculated selection effect in contrast to the term plant legacy effect which tests the origin community in the Jena experiment). The model-predicted mean value was used for *Poa pratensis* monocultures for monoculture plant legacy. Net effect, complementarity effect and selection effect values were also analyzed using mixed-model analysis of variance. Here, the fixed terms were plant legacy (monoculture vs. mixture selection communities), soil treatment (neutral vs. soil legacy) and their interaction. Species composition was used as random term.

Finally, we calculated soil-feedback effects as the log-ratio of the pot community biomass achieved in neutral soil vs. inoculated soil. Fixed terms were planted diversity (monoculture vs. mixture test communities), plant legacy (monoculture vs. mixture selection communities) and their interaction. Species composition was used as random term.

Results

Biodiversity Effects

As expected, mixed species test communities were more productive than monoculture test communities (Table 1, $P = 0.019$). Test communities with soil legacy tended to have higher biodiversity net effects than communities with neutral soil (Figure 1a; Table 2, $P = 0.076$) indicating positive soil-legacy strengthen positive plant diversity effects. Indeed, soil legacy significantly increased the biodiversity complementarity effect (Table 2, $P = 0.002$). However, this main effect of soil legacy was driven by an underlying significant interaction

between plant- and soil-legacy effects (Figure 1b; Table 2, $P = 0.008$): the complementarity effect was particularly large in potted mixtures assembled from progeny derived from mixture plots in the Jena Experiment and containing the corresponding soil inoculum from the same plots. In contrast, biodiversity selection effects were reduced by soil legacy (Table 2, $P = 0.021$), again driven by an underlying plant- by soil-legacy interactions: the selection effect was particularly low (even negative) in potted mixtures assembled from progeny derived from mixture plots containing the corresponding soil inoculum (Figure 1c; Table 2, $P = 0.006$).

Plant–Soil Feedbacks

In spite of the high complementarity effects for potted communities with mixture plant legacy and soil legacy, on average the biomass of potted communities with mixture plant legacy was negatively affected by soil legacy, whereas the opposite was the case for the biomass of potted communities with monoculture plant legacy (Table 1, $P = 0.02$). Mixture types (plants with mixture legacy, i.e. selected in mixtures) experienced overall negative plant–soil feedbacks, while monoculture types experienced overall positive plant–soil feedbacks (Figure 2a; Table 3, $P = 0.002$). However, the negative effect of soil inoculum was much stronger for mixture types when the soil inoculum came from monoculture plots in the Jena Experiment, i.e. when plant and soil legacy did not match ("away" soil), than when inoculum came from mixture plots (see in particular the two species *Crepis biennis* and *Onobrychis viciifolia* in Fig. 2b). Here it must be noted that away-soil combinations occurred in potted monocultures containing plants derived from mixture plots in the Jena Experiment and in potted mixtures containing plants derived from monoculture plots; the other two combinations reflected home-soil combinations. Monoculture types (plants with monoculture legacy, i.e. selected in monocultures) experienced positive soil-feedbacks independent of whether the soil legacy was of monoculture or mixture plots in the Jena Experiment (Table 3, $P = 0.046$). Thus, overall potted communities with monoculture plant legacy seemed to benefit most from soil legacy feedbacks. Only for two of the eight tested species did soil

legacy have a negative feedback on plants with monoculture legacy (*Crepis biennis*, *Trifolium repens*, Figure 2b).

Weed Biomass in Monocultures vs. Mixtures

Mixture test communities had a lower proportional weed biomass than monoculture test communities (Appendix B: Figure 1; Table 1, $P = 0.012$). This effect was stronger if plants came from mixture plots in the Jena Experiment and were inoculated with mixture legacy soil from the Jena Experiment (Appendix B: Table 1, $P = 0.018$). Soil with monoculture legacy inoculum reduced the weed resistance of monocultures composed of mixture type plants in contrast to monoculture type plants.

Discussion

Our results suggest that not only is grassland primary productivity driven by selection for increased resource complementarity in plant–plant interactions, but that such processes are integrated with plant–soil feedbacks. Individual plant performance may be enhanced by plant community diversity (Schmidtke et al. 2010) as well as by soil feedbacks which could promote coexistence in plant communities (Klironomos 2002). Such interactions can contribute to positive biodiversity–productivity relationships in grasslands (Kulmatiski et al. 2012).

The positive biodiversity effect on productivity that we observed in our potted plant communities has been widely observed in grasslands (Balvanera et al. 2006, Isbell et al. 2009). However, here we could demonstrate for the first time that the biodiversity–productivity relationship was influenced by plant- and soil-legacy effects in the way that plants selected for 8 years in mixture plots in a biodiversity experiment and grown on substrate inoculated with 8-year old soil from these plots showed a particularly strong biodiversity complementarity effect. This suggests that complementarity between plant species in mixtures can increase over time due to the selection of particular plant types and

due to the selection of particular communities of soil organisms. In the case of plant-legacy effects, selection refers to selection of different plant phenotypes in mixtures vs. monocultures (the alternative plant legacy in our study). These may be reflecting different genotypes or epigenetic variation or differential, persistent maternal carry-over effects. Considering the high probability that the initial seed material was genetically variable and that all plants were raised from seed under common conditions, we suggest a sorting out process in the Jena Experiment of different-genotypes within sown communities. In the case of soil-legacy effects, selection refers to selection of particular communities of soil organisms in the Jena Experiment as opposed to neutral soil (the non-specific soil-legacy in our study). Thus, short-term plant evolutionary and soil community assembly processes may interact in experimental communities and increase both their productivity and stability over time due to enhanced complementarity effects (Isbell et al. 2009). Such processes may help explain the increased complementarity effects observed over time in many biodiversity studies (see e.g. Cardinale et al. 2007, Reich et al. 2012). To which extent these processes involve the reduction of negative or increase of positive associations between plants and soil organisms remains unclear. In some cases mutualistic soil microbes have been shown to reduce competition between species (Wagg et al. 2011), perhaps by increasing the productivity of less dominant species (van der Heijden et al. 1998), thereby enhancing productivity in species rich plant communities (Eisenhauer 2012).

In parallel with the increased complementarity effect in mixtures with plant and soil legacy, the selection effect decreased for plant communities composed of plants with mixed species plant legacy grown in soil with a mixed species legacy. This is consistent with previous studies which found that selection effects tend to decrease over time in mixed species communities (Cardinale et al. 2007, Isbell et al. 2009). Low and even negative selection effects for mixture legacy individuals planted in mixed test communities suggest pathogen control of dominant species (Klironomos 2002) implying that communities may be

regulated in part by negative plant–soil feedbacks (Bever 1994, Mills and Bever 1998). We demonstrated for the first time such feedbacks may contribute to the positive effect of biodiversity on plant community productivity as has previously been suggested (Schnitzer et al. 2011, Kulmatiski et al. 2012).

In our study, negative plant–soil feedbacks for plants with mixture legacy (mixture types) were smaller in potted mixtures with mixture soil legacy than in potted monocultures with monoculture soil legacy (see Fig. 2b). This suggests that there may be coadaptation between plants and soil organisms as has been observed in other studies (Pregitzer et al. 2010, Cook-Patton et al. 2011). The negative plant–soil feedbacks for mixture types in monocultures suggests that the inoculum from 8-year old monoculture plots may have contained a large amount of species-specific pathogens (Petermann et al. 2008) to which mixture types had little resistance (mismatch between plant and soil legacy). This suggests mixture types may invest in increased growth at the cost pathogen defense. In contrast, monoculture types in our study benefitted from positive plant–soil feedbacks and this especially in monocultures, where the soil inoculum came from exactly the same plot as the plants (matched plant and soil legacy). We would have expected at least that they would suffer less than mixture types, but that they did even better in soils from the Jena Experiment cannot simply be explained by increased resistance to species-specific pathogens (Petermann et al. 2008, Schnitzer et al. 2011) but hints towards a better use of mutualists by plants with monoculture legacy in soils with monoculture legacy (or even mixture legacy, see monoculture types potted in mixtures with inoculated soils in Fig. 2b).

Increased species and functional diversity can lead to a greater resistance to invasibility in plant communities (Fargione et al. 2003, Jiang et al. 2007). Furthermore, we found that mixture types were consistently more resistant to weed infiltration with the exception of in test monocultures grown in monoculture legacy soil. Progeny of plants

selected in mixtures may therefore be selected for increased resistance to weeds over time. The lower weed resistance we observed when mixture legacy plants were planted in monoculture test communities with soil legacy appears to be the result of reduced biomass production arising from a more negative plant–soil feedback.

Our study suggests that in experimental plant communities of low vs. high diversity differential selection of particular plant phenotypes and of particular communities of soil organisms leads to plant- and soil-legacy effects that may explain increased biodiversity effects over time often observed in biodiversity experiments. This was indicated by the increased complementarity and decreased selection effect in potted mixtures with plant and soil legacy. Positive complementarity and negative selection effects also increase species coexistence and may thus enhance stability as well as productivity. The results of this study emphasize the potential importance of time for community assembly and the development of biodiversity effects under experimental conditions. If similar processes occur in natural systems, older communities may be more stable and have higher productivity than younger communities of similar species composition, with obvious consequences for ecosystem restoration. By understanding mechanisms whereby primary producers coexist and drive ecosystem productivity we may better predict the potential effects of species loss on the functioning of natural ecosystems.

Acknowledgements

We thank Daniel Trujillo Villegas, Leigh Oesch and student helpers for assistance with greenhouse and garden work. Thanks to Theres Zwimpfer, Matthias Furler, René Husi and the gardeners of the Jena Experiment for technical assistance. The study was funded by the Swiss National Science Foundation (SNSF grant number 130720 to B. Schmid). G.B. De Deyn acknowledges the NWO-ALW VIDI grant scheme for financial support.

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Table 1: Results of mixed-effects ANOVA for the log-transformed community aboveground biomass 15 weeks after transplanting plants into pots (numDf: degrees of freedom of term, denDf: degrees of freedom of error term [which can be fractional in REML analysis], F statistic, *P*).

Fixed terms	numDf	denDf	F	*P*
Planted diversity; planted monocultures vs. planted mixtures (PD)	1	22.1	6.38	**0.019**
Plant legacy; monoculture history vs. mixture history (PL)	1	59.4	0.29	0.590
Soil treatment; soil with a Jena legacy vs. neutral soil (ST)	1	58.1	0.00	0.960
PD × PL	1	58.6	0.00	0.969
PD × ST	1	57.7	3.84	0.055
PL × ST	1	58.3	5.76	**0.020**
PD × PL × ST	1	57.8	1.49	0.227
Random terms	VC	s.e.		
Species combination (Sp.comb)	0.0056	0.0093		
Block (B)	0.5938	0.1921		
B × Sp.comb	0.0278	0.0196		
Sp.comb × PL × ST	0.0496	0.0250		
Residual	0.2200	0.0275		

Table 2: Results of mixed-effects ANOVA for biodiversity effects net effects (NE), complementarily effects square-root transformed (CE), selection effects square-root transformed (SE); numDf: degrees of freedom of term, denDf: degrees of freedom of error term [which can be fractional in REML analysis], F statistic, *P*).

NE				
Fixed terms	numDf	denDf	F	*P*
Plant legacy; monoculture history vs. mixture history (PL)	1	160.3	0.89	0.346
Soil treatment; soil with a Jena legacy vs. neutral soil (ST)	1	157.1	3.19	0.076
PL × ST	1	157.1	0.14	0.710
Random terms	VC	s.e.		
Species combination	159.1	64.9		
Residual	202.1	22.8		
CE				
Fixed terms	numDf	denDf	F	*P*
Plant legacy; monoculture history vs. mixture history (PL)	1	161.2	1.69	0.196
Soil treatment; soil with a Jena legacy vs. neutral soil (ST)	1	157.2	8.09	**0.005**
PL × ST	1	157.2	3.98	**0.048**
Random terms	VC	s.e.		
Species combination	4.161	1.781		
Residual	7.771	0.877		
SE				
Fixed terms	numDf	denDf	F	*P*
Plant legacy; monoculture history vs. mixture history (PL)	1	14.1	0.13	0.726
Soil treatment; soil with a Jena legacy vs. neutral soil (ST)	1	14.7	6.74	**0.021**
PL × ST	1	137.4	7.85	**0.006**
Random terms	VC	s.e.		
Species combination (Sp.combo)	1.314	0.974		
Sp.combo × PL	0.096	0.520		
Sp.combo × ST	0.722	0.746		
Residual	7.014	0.872		

Table 3: Mixed-effects ANOVA for soil-feedbacks (log-ratio of values in neutral soil vs. inoculated soil) calculated from mean log-transformed ratio aboveground dry biomasses of plant communities (numDf: degrees of freedom of term, denDf: degrees of freedom of error term [which can be fractional in REML analysis], F statistic, *P*).

Fixed terms	numDf	denDf	F	*P*
Planted diversity; planted monocultures vs. planted mixtures (PD)	1	21.2	1.79	0.195
Plant legacy; monoculture history vs. mixture history (PL)	1	113.9	10.27	**0.002**
PD × PL	1	112.0	4.06	**0.046**
Random terms	VC	s.e.		
Species combination	0.070	0.049		
Residual	0.494	0.067		

Figure 1: Biodiversity effects were assessed by additive partitioning to b) net effect partitioned into c) complementarity effect and d) selection effects (SEM) for monoculture legacy and mixture legacy plants grown in a neutral soil or in full soil legacy inoculum collected from the respective monoculture or mixed species plots in the Jena Experiment, Germany.

Figure 2: Values for soil-feedbacks (log-ratio of values in Jena legacy soil/ neutral soil. , SEM) calculated from a) overall mean log-transformed community aboveground biomass. The increased negative plant–soil feedback for plants selected for in mixtures was observed in most of the species as calculated by the mean log-transformed aboveground biomasses of plant species b) selected for in monoculture and mixture communities for eight years grown in test experimental monocultures (Mono) and mixtures (Mix). The species are arranged according to functional groups: grasses, small herbs, tall herbs and legumes.

Figure 1

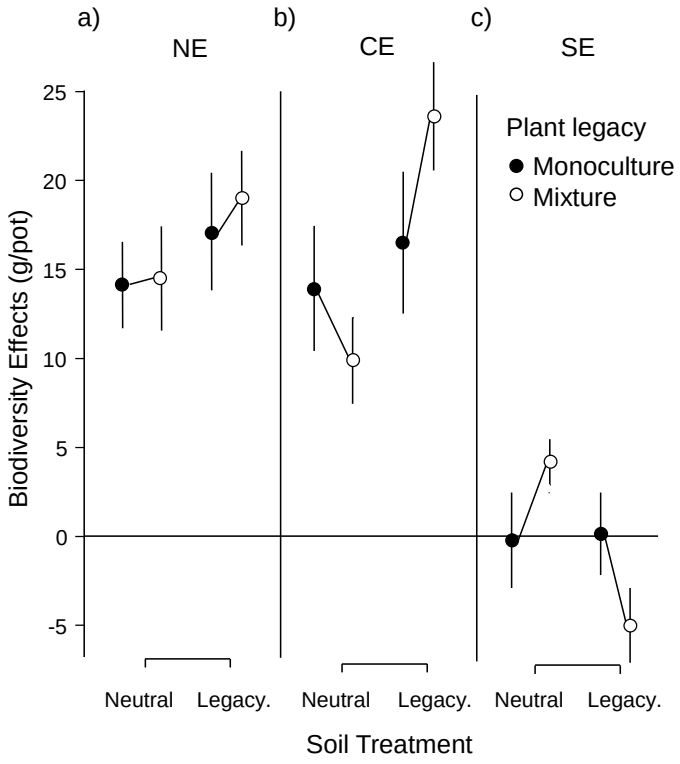
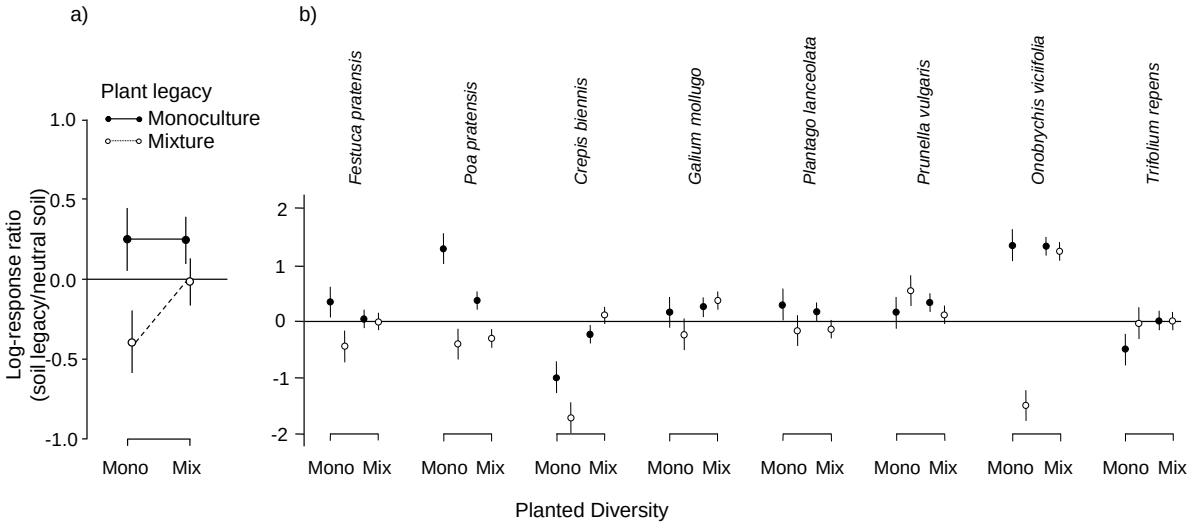

a)
NE
b)
CE
c)
SE
Plant legacy
Monoculture
Mixture
Biodiversity Effects (g/pot)
25
20
15
10
5
0
-5
Neutral
Legacy.
Neutral
Legacy.
Neutral
Legacy.
Soil Treatment

Figure 2


a)
b)
Plant legacy
Monoculture
Mixture
Log-response ratio
(soil legacy/neutral soil)
1.0
0.5
0.0
-0.5
-1.0
Mono
Mix
Festuca pratensis
Poa pratensis
Crepis biennis
Galium mollugo
Plantago lanceolata
Prunella vulgaris
Onobrychis viciifolia
Trifolium repens
2
1
0
-1
-2
Mono Mix
Planted Diversity

Supplemental Material

Appendix A: Supplemental Methods

Table S1: Four-species combinations of each of the 8 species from monoculture or mixture legacy communities established in the Jena Experiment. Fes pra = *Festuca pratensis*, Poa pra = *Poa pratensis*, Pla lan = *Plantago lanceolata*, Pru vul = *Prunella vulgaris*, Cre bie = *Crepis biennis*, Gal mol = *Galium mollugo*, Ono vic = *Onobrychis viciifolia*, Tri rep = *Trifolium repens*.

Grass	Short herb	Tall herb	Legume
Fes pra	Pru vul	Gal mol	Tri rep
Fes pra	Pru vul	Gal mol	Ono vic
Fes pra	Pru vul	Cre bie	Tri rep
Fes pra	Pru vul	Cre bie	Ono vic
Fes pra	Pla lan	Gal mol	Tri rep
Fes pra	Pla lan	Gal mol	Ono vic
Fes pra	Pla lan	Cre bie	Tri rep
Fes pra	Pla lan	Cre bie	Ono vic
Poa pra	Pru vul	Gal mol	Tri rep
Poa pra	Pru vul	Gal mol	Ono vic
Poa pra	Pru vul	Cre bie	Tri rep
Poa pra	Pru vul	Cre bie	Ono vic
Poa pra	Pla lan	Gal mol	Tri rep
Poa pra	Pla lan	Gal mol	Ono vic
Poa pra	Pla lan	Cre bie	Tri rep
Poa pra	Pla lan	Cre bie	Ono vic

Appendix B: Supplemental Results

The number of individuals surviving in our experimental communities did not indicate differential survival for mixture and monoculture types over the 15 weeks of our study (Table S2).

Table S1: Results of mixed-effects ANOVA for weed biomass proportional to community biomass (numDf: degrees of freedom of term, denDf: degrees of freedom of error term [which can be fractional in REML analysis], F statistic, *P*).

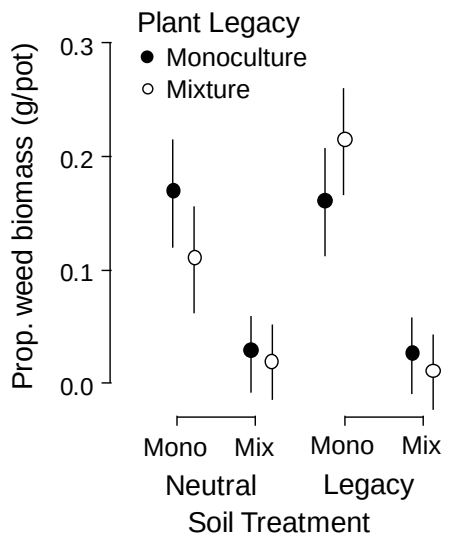
Fixed terms	numDf	denDf	F	*P*
Planted diversity; planted monocultures vs. planted mixtures (PD)	1	22.1	7.59	**0.012**
Plant legacy; monoculture history vs. mixture history (PL)	1	59.0	0.36	0.550
Soil treatment; soil with a Jena legacy vs. neutral soil (ST)	1	22.1	0.88	0.357
PD × PL	1	58.6	0.12	0.728
PD × ST	1	22.0	3.15	0.090
PL × ST	1	33.2	2.45	0.127
PD × PL × ST	1	32.9	6.22	**0.018**
Random terms	VC	s.e.		
Species combination (Sp.comb)	0.013	0.004		
Sp.comb × ST	0.001	0.001		
Sp.comb × ST × PL	0.002	0.001		
Sp.comb × PL × Block	0.002	0.001		
Residual	0.003	0.001		

Table S2: Results of mixed-effects ANOVA for survival of plant individuals per pot (numDf: degrees of freedom of term, denDf: degrees of freedom of error term [which can be fractional in REML analysis], Wald statistic, F statistic, *P*).

Fixed terms	numDf	denDf	F	*P*
Planted diversity; planted monocultures vs. planted mixtures (PD)	1	41.9	2.08	0.157
Plant legacy; monoculture history vs. mixture history (PL)	1	41.3	1.24	0.271
Soil treatment; soil with a Jena legacy vs. neutral soil (ST)	1	22.1	1.21	0.283
PD × PL	1	41.3	1.4	0.243
PD × ST	1	21.7	0.96	0.338
PL × ST	1	114.9	1.48	0.227
PD × PL × ST	1	112.9	0.07	0.793
Random terms	VC	s.e.		
Block (B)	0.003	0.008		
Sp.comb × PL	2.861	0.646		
Sp.comb × ST	0.024	0.028		
B × Sp.comb × PL	0.030	0.041		
Residual	0.365	0.049		

Figure S1: Weed biomass proportional to pot biomass (SEM) of plant communities selected for in and mixture communities for 8 years grown in new experimental monoculture (Mono) and mixtures (Mix) in either a neutral soil substrate inoculated with either sterilized inocula or with full soil inocula collected from respective monoculture plots or mixed species plots in the Jena experiment, Germany.

Figure S1


Plant Legacy
Monoculture
Mixture
Prop. weed biomass (g/pot)
0.3
0.2
0.1
0.0
Mono
Mix
Mono
Mix
Neutral
Legacy
Soil Treatment

Chapter Three

(Formatted for submission to Journal of Plant Ecology)

Selection in monoculture vs. mixture alters plant metabolic fingerprints

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Running title: Fingerprint of selection in a biodiversity experiment

Key words: biodiversity; plant evolution; selection; FTIR spectroscopy; metabolic fingerprint

Abstract

Aims In grassland biodiversity experiments, positive biodiversity effects on primary productivity increase over time. Recent research has shown that differential selection in monoculture and mixed-species communities leads to the rapid emergence of monoculture and mixture types, adapted to their own biotic community. We used eight plant species selected for 8 years in such a biodiversity experiment to test if monoculture and mixture types differed in metabolic profiles using infrared spectroscopy.

Methods Fourier transform infrared spectroscopy (FTIR) was used to assess metabolic fingerprints of leaf samples of 10 individuals of each species from either monocultures or mixtures. The FTIR spectra were analyzed using multivariate procedures to assess 1) whether individuals within species could be correctly assigned to monoculture or mixture history based on the spectra alone and 2) which parts of the spectra were responsible for the group assignation, i.e. which metabolic groups were subject to differential selection in monocultures vs. mixtures.

Important Findings Plant individuals within each of the eight species could be classified as either from monoculture or mixture selection history based on their FTIR spectra. Different metabolic groups were differentially selected in the different species; some of them may be related to defense of pathogen accumulating more strongly in monocultures than in mixtures. The rapid selection of the monoculture and mixture types within the eight study species could have been due to a sorting-out process based on large initial genetic variation within the species.

Introduction

Greater biodiversity in plant communities positively affects productivity and this effect can increase over time (Reich *et al.*, 2012, Tilman *et al.*, 2006). Positive biodiversity effects on productivity are often interpreted in terms of more complementary resource uptake (HilleRisLambers *et al.*, 2004, Schnitzer *et al.*, 2011, Tilman *et al.*, 2001) as plants separate along environmental niche axes (Silvertown, 2004). Additionally, plant–soil feedbacks can contribute to positive biodiversity effects on productivity by regulating plant species co-existence in plant communities (Bever, 1994, Bever, 2003, Mills et al., 1998, Petermann *et al.*, 2008), leading to lower productivity at the lower end of the biodiversity gradient (Kulmatiski *et al.*, 2012, Schnitzer, Klironomos, HilleRisLambers, Kinkel, Reich, Xiao, Rillig, Sikes, Callaway, Mangan, van Nes and Scheffer, 2011). In addition, recent work has demonstrated that plants can become adapted to the diversity of the community in which they grow (Lipowsky *et al.*). Such selection processes can influence productivity, as reflected by cultivation of individuals grown in monocultures or mixed species plots, where productivity in experimental mixtures or monocultures was determined by the origin community (Zuppinger-Dingley *et al.*, in prep., Zuppinger-Dingley *et al.*, in prep.). These new findings indicate the potential for plant adaptation to the biotic environment; and, consequently, should be reflected in phenotypic differences among the selected low- and high-diversity or monoculture and mixture plant types. In this study we assessed metabolic fingerprints of leaf tissues with Fourier transform infrared spectroscopy (FTIR) as an indication of such phenotypic differences. We used eight grassland plant species which had been growing for 8 years in monoculture or mixed-species communities in a large grassland biodiversity experiment in Jena, Germany. We refer to the two types of communities as selection histories.

Plant individuals have been shown to adapt to the local biotic environment in a study focusing on plant traits across the experimental plant species richness gradient of this Jena Experiment (Lipowsky, Schmid and Roscher, 2011). The phenotypic changes in response to

the local biotic environment could have been due to genetic, epigenetic or maternal effects (Roach *et al.*, 1987, Rossiter, 1996). Fourier transform-infrared spectroscopy (FTIR) is a promising tool to determine phenotypic changes at the level of plant biochemistry. Although this technique is not often used in plant community ecology (Gidman *et al.*, 2003), FTIR fingerprinting has been used successfully to differentiate between plant genera (Gorgulu *et al.*, 2007), to identify major alterations in biochemical pathways of mutant collections (Sardans *et al.*, 2011) and to determine metabolic alterations within species subjected to changes in environmental conditions (Domenighini *et al.*, 2009, Gidman, Goodacre, Emmett, Smith and Gwynn-Jones, 2003, Harmanescu *et al.*, 2012, Jones *et al.*, 2012, Lazar *et al.*, 2012, Scherling *et al.*, 2010). It is conceivable that it is possible to link such changes in biochemical pathways with underlying genetic alterations (Fiehn, 2002).

FTIR produces a biochemical signature of a selected sample (Fiehn, 2001, Johnson *et al.*, 2003) providing a snapshot of the biochemical composition of a cell (Domenighini and Giordano, 2009) produced as a metabolic fingerprint used to discriminate not only between species but also between genotypes within species (Schulz *et al.*, 2007). Furthermore, because absorption peaks in FTIR spectra are due to the particular chemical bonds making up materials (Ammann *et al.*, 2011), there is the possibility to assign peaks to particular groups of compounds such as nucleic acids, lipids and carbohydrates (Griffiths *et al.*, 1986).

Method

The Jena Experiment was our source of plants from communities with a selection history of either monoculture (monoculture types) or mixed-species diversity (mixture types) in the field. This experiment was established in 2002 at a field site in Jena, Germany (50°55'N, 11°35'E, 130 m a.s.l), using 60 common Central European grassland species. Sown plant species richness ranged from 1–60 species per plot (see (Roscher *et al.*, 2004) for details). We chose eight of the 60 species based on their occurrence both in plots of

monocultures or mixtures for 8 years in the Jena Experiment to test if metabolic changes (changes in biochemical composition) had occurred in response to selection in monocultures vs. mixtures. Two species from each of four plant functional groups were chosen: grasses (*Festuca pratensis*, *Poa pratensis*), legumes (*Onobrychis viciifolia*, *Trifolium repens*), tall herbs (*Crepis biennis*, *Galium mollugo*) and small herbs (*Plantago lanceolata*, *Prunella vulgaris*). We collected plant cuttings from the experimental plots in Jena in April 2010 and replanted them into pots with GVZ Tref GO PP 7000 (BF4; GVZ; De Baat, Holland) substratum under glasshouse conditions to acclimatize them to the new environment before transplanting them into plots in our experimental garden at University of Zurich with the same species combinations as found in the original Jena plots. We propagated the plant material as cuttings of cuttings, so that the material used in the study was not directly taken from the field.

Using a JASCO 4200-FTIR instrument (Brechbühler AG, Schlieren, Switzerland) in attenuated total reflection (ATR) mode with an ATR accessory equipped with a zinc selenide (ZnSe) prism, we measured metabolic fingerprints. Whole mature leaf samples from randomly were taken from plant individuals of the study species and placed onto the ATR accessory and spectra were collected (Hsu, 1997). For each leaf sample an average of 50 scans were taken with a resolution of 4 cm^{-1} using the ZnSe prism and saved for further chemometrical analyses. We used a measurement range of 650–4000 cm^{-1}. For each study species leaf samples were taken from 10 individuals derived from 10 cuttings of different plants selected in monocultures and the same number were taken from individuals derived from plants selected in mixtures. Raw spectral data were processed with JASCO Spectra Manager 2.02.02. Each spectrum was adjusted using baseline correction (linear), ATR-correction, smoothing (Savitzky-Golay, width = 15; (Susi *et al.*, 1983), truncation (1900–650 cm^{-1}) and (v) normalization (highest value =1, lowest value = 0).

Linear discriminant analysis (LDA; Ripley, 1994), was used to calculate classification functions and assign leaf samples to their respective selection history (monocultures vs. mixtures) in a single analysis of all species (R, version 2.15.3, R Development Core Team, 2013). Canonical variates analysis (Hotelling, 1936) was used to estimate multivariate intergroup distances for each species with the Mahalanobis D-squared distance measure (CVA; with GenStat version 16, VSN International Ldt. 2013). We performed stepwise multiple regression (Hocking, 1976) to determine the selected wavenumber regions that drove the differences between monoculture and mixture types for all species in a single analysis (R, version 2.15.3, R Development Core Team, 2013). Multidimensional scaling (MDS), a multivariate method for data visualization of hidden relations among objects in data (Borg *et al.*, 2005), in the form non-metric multidimensional scaling (NMDS), was applied on the combined spectral range to determine dissimilarities among samples between each selection history ("Q-mode" analysis) for each individual species and in a single analysis of all species (R, version 2.15.3, R Development Core Team, 2013). Analysis was performed on the following spectral regions broadly assigned to four groups of compounds:

Aromatic = 650–910 cm^{-1} (Hsu *et al.*, 1997),

Carbohydrate = 750–1200 cm^{-1} (Ami *et al.*, 2013)

Protein = 1500–1700 cm^{-1} (Amiali *et al.*, 2011)

Lignin = 1590–1610 cm^{-1} (Allison, 2011).

We focused on these spectral regions to determine if specific wavenumber regions could be associated with observed classification into monoculture or mixture selection history. Applying the two orthogonal ordination axes from the NMDS analysis of all species with selection history as binary response variable in generalized mixed models (Breslow *et al.*, 1993, Wolfinger *et al.*, 1993); GenStat ,version 16 ,VSN International Ldt. 2013), we tested if plants selected in either monoculture or mixture communities over 8 years showed distinct metabolic fingerprints. The results were summarized in analyses of variance (ANOVA)

tables. Significance tests were based on approximate F-tests using appropriate error terms and denominator degrees of freedom. The fixed terms in the models were: selection history (monocultures vs. mixtures), species and interactions among these. Species and plant individual were used as random terms.

The second derivative of the corrected spectra, allowing for band narrowing and therefore distinguishing more features, was then calculated (Savitzky-Golay, width = 15; Susi and Michael Byler, 1983). Hierarchical cluster analysis, using the complete linkage method with Euclidean distance (Everitt, 1974, Hartigan, 1975); R, version 2.15.3, R Development Core Team, 2013), was used to determine which samples were most alike and therefore would cluster together and how well these clusters represented the selection history of the species.

Results

Selection history clearly altered the metabolic fingerprints of the species in our study. The matrix produced using LDA showed that plant individuals were 99% correctly classified as belonging to either monoculture or mixture selection history (Table 1). Two of the species, *Plantago lanceolata* and *Poa pratensis,* accounted for the 1% failure in the classification of individuals into monoculture or mixture selection history. Using single wavenumber groups related to the compounds proteins, carbohydrates and aromatics (Fig. 1) we obtained similar levels of accuracy, i.e. 99% correct classification into monoculture or mixture selection history. However, the wavenumber group associated with lignin (Fig. 1; Table S1) showed the least number of individuals correctly classified into the respective selection history. Certain specific wavenumbers with significantly different absorption between monoculture and mixture plant types could tentatively be associated with specific biochemical compounds (Table 2).

Additional evidence for a shift in metabolic fingerprints with selection history was provided by the separate analyses for each species using Mahalanobis distances for the four

wavenumber groups mentioned above, showing that the maximum distance was always between plant individuals from different selection histories (Table 3; Fig. S1). The greatest distance between the selection histories was found across all wavenumbers combined and in the protein wavenumber region in *Prunella vulgaris*; in the aromatic and lignin wavenumber regions *Trifolium repens* and *Onobrychis viciifolia* showed the greatest distance, respectively. In the carbohydrate wavenumber region the greatest distance between the selection histories occurred in *Galium mollugo*.

In addition, NMDS-ordinations based on Euclidean distance dissimilarities calculated between the 20 individuals of each of the eight species separately showed that individuals with monoculture selection history were clearly separated from individuals with mixture selection history in the ordination plots for the eight species (Fig. 2). Stress values of under 0.075 indicate a high degree of correspondence between the observed inter-object distances and the distances predicted by the dissimilarities. Mixed effects models using the combined data set of all eight species (Table 4) showed that all species differed significantly in their FTIR spectra (P = <0.001). Differences between monoculture and mixture selection history were in part common to all species (significant main effects of selection history in Table 4) but additionally highly species-specific (significant interactions in Table 4). These results show 8 years of selection in monocultures vs. mixtures has led to clearly differentiated metabolic fingerprints in the eight studied grassland species.

Finally, cluster analysis on the second derivative of spectra for each species clearly differentiated between monoculture types and mixture types for most species, with individuals of common selection history generally clustering together (Fig. 3; Fig. S2). Monoculture types clustered particularly strongly into single groups in the two tall herbs *Galium mollugo* and *Crepis biennis* and in the two legumes *Onobrychis viciifolia* and *Trifolium repens*. In contrast, mixture types clustered particularly strongly in the two grasses *Festuca pratensis*

and *Poa pratensis*. The small herb *Prunella vulgaris* showed the weakest separation between monoculture and mixture types whereas the other small herb *Plantago lanceolata* showed stronger clustering for monoculture history.

Discussion

We determined metabolic alterations occurring after eight years of selection in plant communities of monocultures or mixed-species diversity in the Jena Experiment, Germany. These metabolic alterations show that plants with different biochemical features have been selected in monoculture vs. mixed-species communities. Currently, we cannot say whether the response to selection was based on different plant genotypes occurring in the populations of the study species or if the phenotypic differences reflect differential epigenetic or maternal carryover effects. Independent of the mechanisms, it also appeared that selection was stronger in monocultures than in mixtures because clustering of spectra was tighter among plant individuals with monoculture than with mixture selection history. This may have been related to larger population sizes in experimental plots harboring only one rather than several species or to stronger selection pressures exerted e.g. by pathogen accumulation in monocultures (Magarey, 1999, Petermann, Fergus, Turnbull and Schmid, 2008).

Similar alterations in metabolic responses of vascular plants to environmental conditions have been reported. IR spectroscopy identified metabolic differences in tomato fruits from plants that were grown either under normal conditions or subject to salinity stress (Johnson, Broadhurst, Goodacre and Smith, 2003). Additionally, tomato plants showed metabolic alterations of leaf tissue in response to nitrogen nutrition under two different light intensities (Urbanczyk-Wochniak *et al*., 2005). In grassland plants, alterations in metabolic fingerprints have been observed in response to different types of fertilizer (Harmanescu, Alexandru and Gergen, 2012). Furthermore the effects of grazing on the grass *Deschampsia*

flexuosa could be detected in its metabolic fingerprints (Jones, Causton, Emmett, Mur and Gwynn-Jones, 2012).

The above studies mainly reflect plastic responses of genotypes to growth in different environments, whereas in our study they reflect differences among plants derived from different selection environments but grown in a common environment. This indicates that the described changes can also be heritable, although this heritability may be due to genetic or epigenetic differences or to persistent maternal carry-over effects. Recently, evidence for variation in metabolic patterns associated with species richness was found for three plant species, *Lotus corniculatus*, *Bellis perennis* and *Leontodon autumnalis*, after six years in the Jena Experiment in plots of 1, 2, 4, 8, 16 and 60 species (Scherling, Roscher, Giavalisco, Schulze and Weckwerth, 2010). Although in this case the plants were observed *in situ* in the different biotic environments, these results are consistent with our findings. Thus, for those three species it is conceivable that the responses reflected more than plastic adjustments of individual genotypes, namely selection of different genotypes in the different environments.

Genetically-caused differences in FTIR fingerprints have been reported between *Arabidopsis thaliana* mutants and wild-types (Fiehn, 2002) and in the same species metabolomics have been used to differentiate genotypes (Macel *et al.*, 2010). In a study with *Acantholimon*, *Astragalus*, and *Ranunculus* species, not only were clusters representing the three genera produced using FTIR fingerprints of their leaves, but also such fingerprints differentiated subgroups of species according to the source geographical regions (Gorgulu, Dogan and Severcan, 2007). Similarly, in a field study using the Douglas–fir, *Pseudotsuga menziesii*, a strong signal environmental variation could be shown in metabolite profiling, despite weak signal of genetic variation (Robinson *et al.*, 2007).

Although we tentatively assigned compounds to the wavenumbers significantly contributing to variation between the two selection histories of monoculture vs. mixed-species

communities, further studies are needed to identify specific compounds underlying the possible adaptations to the specific biotic environments. Furthermore despite hypothesizing that the differences we observed may be due to epigenetic or maternal effects, we suggest that they are more likely based on differential selection of genotypes. This would imply that the original plant material used to establish the Jena Experiment contained a large amount of standing genetic variation from which pre-adapted genotypes could be selected by a sorting process. Together with recombination events during sexual reproduction this would then have allowed for the rapid evolution of monoculture and mixture types.

Supplementary Data

We provide additional information on classification of individuals into mixture and monoculture types based on each of the main biochemical classes, aromatics, carbohydrates, protein, and lignins, in Table S1. Figure S1 shows FTIR spectra for each of the eight species, while Figure S2 shows cluster dendrograms for the six species not shown in the main text (see Figure 3).

Acknowledgements

This study was supported by the Swiss National Science Foundation (grant number 130720 to B. Schmid). Thanks to Daniel Trujillo Villegas, Leigh Oesch, Theres Zwimpfer, Matthias Furler, René Husi, the gardeners of the Jena Experiment and student helpers for technical assistance.

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Table 1: LDA matrix of a single analysis of eight grassland species, of the predicted classification into monoculture or mixture selection history using absorbance values in the range of wavenumbers from 650–1900 cm^{-1} derived from FTIR spectroscopy.

Species		*Crepis biennis*		*Festuca pratensis*		*Galium mollugo*		*Onobrychis viciifolia*		*Plantago lanceolata*		*Poa pratensis*		*Prunella vulgaris*		*Trifolium repens*	
	History	Mixture	Monoculture	Mixture	Monoculture	Mixture	Monoculture	Mixture	Monoculture	Mixture	Monoculture	Mixture	Monoculture	Mixture	Monoculture	Mixture	Monoculture
Crepis biennis	Mixture	**9**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Monoculture	**1**	**9**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Festuca pratensis	Mixture	0	0	**10**	**0**	0	0	0	0	0	0	0	0	0	0	0	0
	Monoculture	0	0	**0**	**10**	0	0	0	0	0	0	0	0	0	0	0	0
Galium mollugo	Mixture	0	0	0	0	**10**	**0**	0	0	0	0	0	0	0	0	0	0
	Monoculture	0	0	0	0	**0**	**10**	0	0	0	0	0	0	0	0	0	0
Onobrychis viciifolia	Mixture	0	0	0	0	0	0	**10**	**0**	0	0	0	0	0	0	0	0
	Monoculture	0	0	0	0	0	0	**0**	**10**	0	0	0	0	0	0	0	0
Plantago lanceolata	Mixture	0	0	0	0	0	0	0	0	**10**	**0**	0	0	0	0	0	0
	Monoculture	0	0	0	0	0	0	0	0	**0**	**10**	0	0	0	0	0	0
Poa pratensis	Mixture	0	0	0	0	0	0	0	0	0	0	**10**	**0**	0	0	0	0
	Monoculture	0	0	0	0	0	0	0	0	0	0	**1**	**9**	0	0	0	0
Prunella vulgaris	Mixture	0	0	0	0	0	0	0	0	0	0	0	0	**10**	**0**	0	0
	Monoculture	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**10**	0	0
Trifolium repens	Mixture	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**10**	**0**
	Monoculture	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**10**

Table 2: The most significant wavenumbers in the range 650–1900 cm^{-1} differentiating plant individuals from monoculture vs. mixture selection history in a single analysis of eight grassland species using FTIR, with biochemical compounds tentatively assigned to these wavenumbers (Baseri *et al.*, 2011, Coates, 2000, Movasaghi *et al.*, 2008, Stuart, 1996). All values are significant at $P < 0.001$.

Wave-number (cm^{-1})	Difference in absorbance values	Standard error of difference	*t*-value	Compound
756	-4.31	1.24	-3.47	Aliphatic chloro
804	3.40	0.95	3.60	Left-handed helix DNA
814	-3.70	1.06	-3.48	Epoxy and oxirane rings
872	2.77	0.76	3.65	Epoxy and oxirane rings
881	-4.91	1.07	-4.60	Epoxy and oxirane rings
1016	-5.22	1.39	-3.76	Glycogen
1026	7.58	2.21	3.43	Glycogen
1036	-6.04	1.38	-4.39	Glycogen
1065	5.29	1.40	3.79	Protein amide I
1084	8.44	2.30	3.67	Protein amide I
1257	-11.68	3.18	-3.68	Phospholipids
1267	13.90	3.49	3.98	Phospholipids
1277	-12.00	3.16	-3.80	Phospholipids
1315	-4.99	1.23	-4.05	Aromatic amine
1325	12.21	2.04	5.99	Aromatic amine
1335	-12.82	2.51	-5.11	Polysaccharides, pectin
1431	-9.85	2.50	-3.93	Methylene, methyl groups
1450	10.03	1.71	5.87	Methylene
1460	-4.99	1.15	-4.35	Benzene ring
1489	-10.06	2.32	-4.33	Amide II
1566	17.61	3.80	4.63	Aromatic ring
1576	-13.95	3.53	-3.95	Adenine
1662	16.89	4.50	3.75	Alkenyl (lipids)
1720	-5.15	1.25	-4.10	Ester group
1730	12.25	2.01	6.10	Fatty acid ester
1740	-18.05	3.36	-5.37	Aliphatic ester
1749	15.63	3.18	4.92	Aliphatic ester
1759	-7.68	1.90	-4.03	Alkyl carbonate
1884	-4.54	0.61	-7.50	Carbonyl
1894	4.09	0.57	7.12	Carbonyl

Table 3: Maximum Mahalanobis (D-squared) distance between individuals selected in monocultures vs. mixtures in a biodiversity experiment in Jena, Germany. The eight species were analyzed separately using CVA with absorbance values for wavenumbers in the range 650–1900 cm^{-1} assigned to aromatics (650–910 cm^{-1}), carbohydrate (750–1200 cm^{-1}), protein (1500–1700 cm^{-1}), lignin (1590–1610 cm^{-1}) for the spectral data collected using FTIR spectroscopy.

Species	*Crepis biennis*	*Festuca pratensis*	*Galium mollugo*	*Onobrychis viciifolia*	*Plantago lanceolata*	*Poa pratensis*	*Prunella vulgaris*	*Trifolium repens*
FTIR wavenumber (cm^{-1})								
650 - 1900	17.88	7.81	33.19	17.92	20.34	7.60	26.58	17.88
650 - 910	4.98	8.42	9.08	13.24	4.88	3.94	8.88	23.42
750 - 1200	10.01	7.40	33.19	17.92	4.88	3.29	18.36	4.68
1500 - 1700	5.41	9.88	8.31	14.63	14.63	7.60	23.38	1.82
1590 - 1610	2.59	5.29	6.10	17.98	2.63	5.61	5.79	3.71

Table 4: Results of mixed-effects ANOVA for plants selected in monocultures vs. mixtures over 8 years in a biodiversity experiment in Jena, Germany. MDS axes ("MDS1", "MDS2") calculated from FTIR absorbance values using NMDS analysis were used as dependent variables. They were calculated for the full range of wavenumbers ("Fingerprint") and for four bands of wavenumbers reflecting four categories of biochemical compounds: 650–910 cm^{-1} ("Aromatics"), 750–1200 cm^{-1} ("Carbohydrates"), 1500–1700 cm^{-1} ("Proteins"), 1590–1610 cm^{-1}("Lignins"). Abbreviations: numDf = degrees of freedom of term, denDf = degrees of freedom of error term (which can be fractional in REML analysis), F statistic = variance ratio, *P* = significance level).

MDS1			Fingerprint		Aromatics		Carbohydrates		Proteins		Lignin	
Fixed term	numDf	denDf	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
Species (Sp)	7	72	94.36	<**0.001**	118.27	<**0.001**	56.04	<**0.001**	174.7	<**0.001**	56.04	<**0.001**
Selection history (SH)	1	72	10.97	**0.001**	0.02	0.879	11.73	**0.001**	0.69	0.407	11.73	**0.001**
Sp × SH	7	72	9.83	<**0.001**	2.89	**0.010**	8.91	<**0.001**	11.25	<**0.001**	8.91	<**0.001**
MDS2												
Fixed term	numDf	denDf	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
Species (Sp)	7	72	90.5	<**0.001**	35.31	<**0.001**	185.72	<**0.001**	142.56	<**0.001**	185.72	<**0.001**
Selection history (SH)	1	72	3.53	0.064	1.13	0.291	3.33	0.072	2.50	0.118	3.33	0.072
Sp × SH	7	72	6.27	<**0.001**	8.66	<**0.001**	0.83	0.564	25.54	<**0.001**	0.83	0.564

Figure 1: Mean FTIR spectra wavenumber of eight European grassland species selected in monocultures or mixtures (n = 80 each) over 8 years in a biodiversity experiment in Jena, Germany, showing the variation in the metabolic fingerprint between monoculture and mixture selection history.

Figure 2: NMDS ordination plot based on Euclidean distance dissimilarities of FTIR spectra of leaves from individuals for each of eight central European grassland species selected in monocultures or mixtures over 8 years in a biodiversity experiment in Jena, Germany. Stress values in NMDS analysis reflect a good fitting solution with a high degree of correspondence between the observed inter-object distances and the distances predicted by the dissimilarities; mean linear $R^2 = 0.99$ (based on correlation between fitted values and ordination distances) and mean non-metric fit $R^2 = 0.99$ (based on stress values).

Figure 3: Dendrogram of individuals of two of eight grassland species selected in monocultures or mixtures over 8 years in a biodiversity experiment in Jena, Germany, based on values of the second derivative of FTIR spectra (dendrograms for the other six species are presented in Fig. 2 of the Appendix).

Figure 1

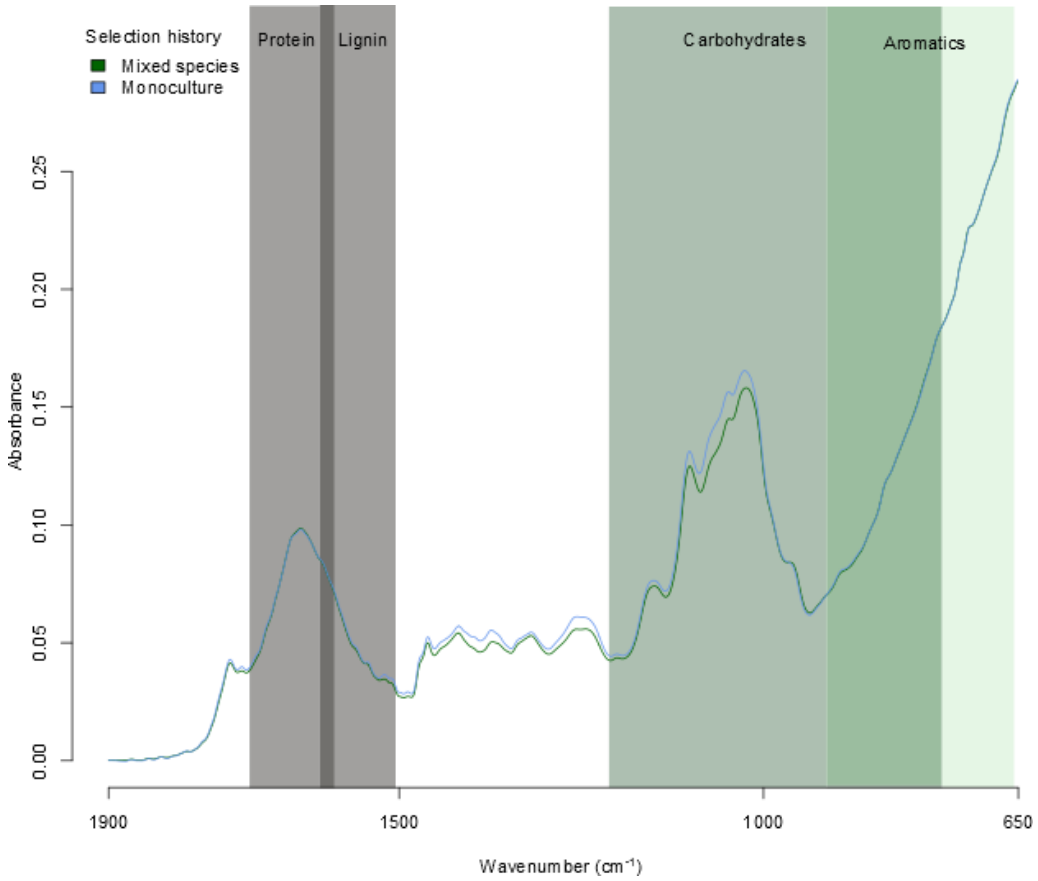

Selection history
Mixed species
Monoculture
Protein
Lignin
Carbohydrates
Aromatics
Absorbance
0.00
0.05
0.10
0.15
0.20
0.25
1900
1500
1000
650
Wavenumber (cm-1)

Figure 2

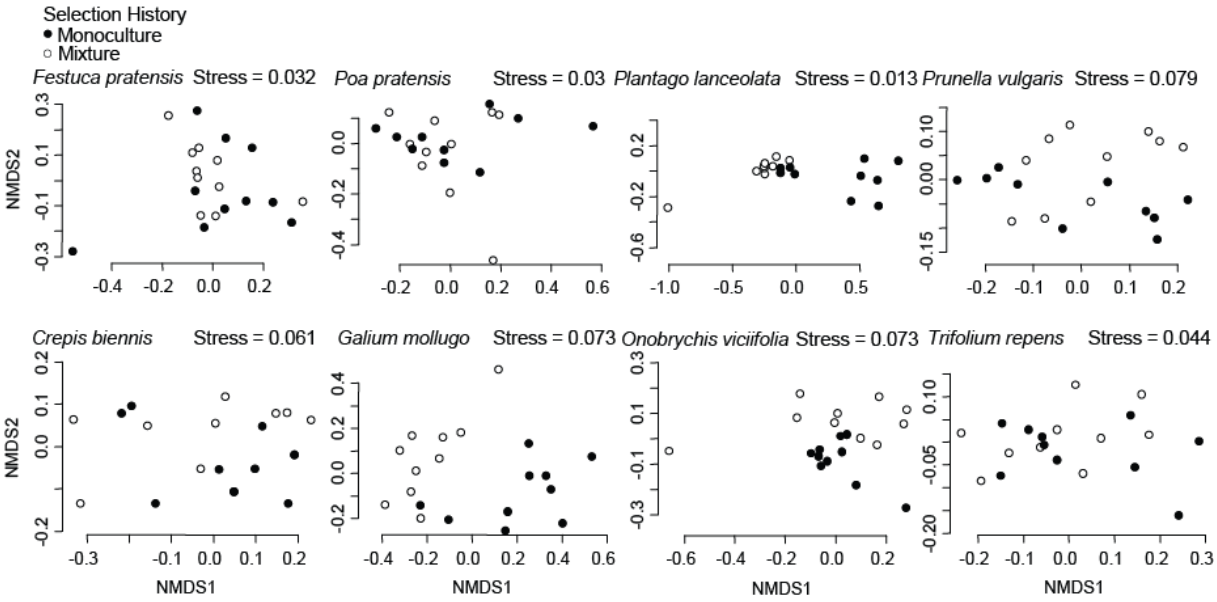
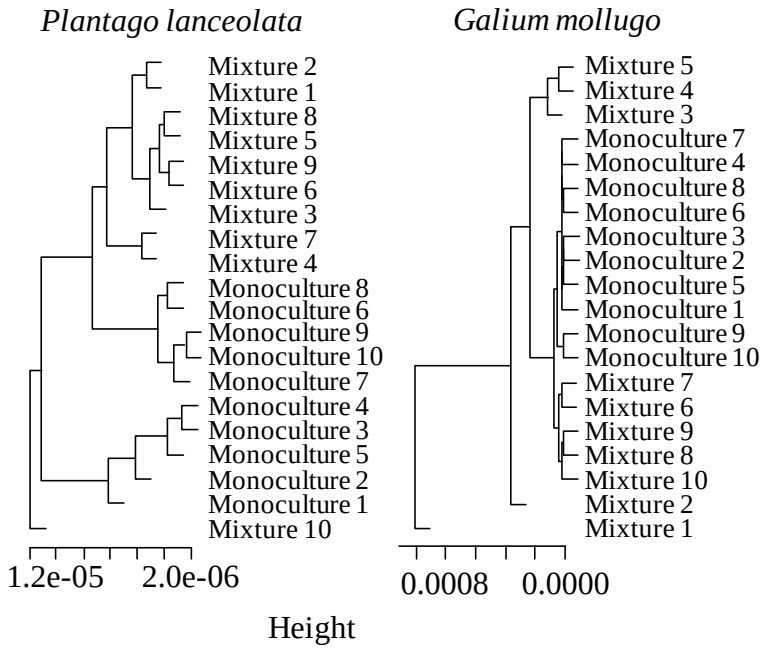

Selection History
Monoculture
Mixture
Festuca pratensis Stress = 0.032
Poa pratensis Stress = 0.03
Plantago lanceolata Stress = 0.013
Prunella vulgaris Stress = 0.079
Crepis biennis Stress = 0.061
Galium mollugo Stress = 0.073
Onobrychis viciifolia Stress = 0.073
Trifolium repens Stress = 0.044
NMDS1
NMDS2

Figure 3


Plantago lanceolata
Mixture 2
Mixture 1
Mixture 8
Mixture 5
Mixture 9
Mixture 6
Mixture 3
Mixture 7
Mixture 4
Monoculture 8
Monoculture 6
Monoculture 9
Monoculture 10
Monoculture 7
Monoculture 4
Monoculture 3
Monoculture 5
Monoculture 2
Monoculture 1
Mixture 10
1.2e-05
2.0e-06
Galium mollugo
Mixture 5
Mixture 4
Mixture 3
Monoculture 7
Monoculture 4
Monoculture 8
Monoculture 6
Monoculture 3
Monoculture 2
Monoculture 5
Monoculture 1
Monoculture 9
Monoculture 10
Mixture 7
Mixture 6
Mixture 9
Mixture 8
Mixture 10
Mixture 2
Mixture 1
0.0008
0.0000
Height

Supplementary Data

Table S1: LDA matrix of a single analysis of eight grassland species with the predicted classification into monoculture or mixture selection history for the FTIR wavenumbers for aromatics (650–910 cm^{-1}), carbohydrate (750–1200 cm^{-1}), protein (1500–1700 cm^{-1}), lignin (1590–1610 cm^{-1}) for the spectral data collected using FTIR spectroscopy with 99%, 99%, 99% and 85% correctly classified respectively.

Species		*Crepis biennis*		*Festuca pratensis*		*Galium mollugo*		*Onobrychis viciifolia*		*Plantago lanceolata*		*Poa pratensis*		*Prunella vulgaris*		*Trifolium repens*	
	History	Mixture	Monoculture	Mixture	Monoculture	Mixture	Monoculture	Mixture	Monoculture	Mixture	Monoculture	Mixture	Monoculture	Mixture	Monoculture	Mixture	Monoculture
Crepis biennis	Mixture	**9**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Monoculture	**0**	**10**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Festuca pratensis	Mixture	0	0	**10**	**0**	0	0	0	0	0	0	0	0	0	0	0	0
	Monoculture	0	0	**0**	**10**	0	0	0	0	0	0	0	0	0	0	0	0
Galium mollugo	Mixture	0	0	0	0	**10**	**0**	0	0	0	0	0	0	0	0	0	0
	Monoculture	0	0	0	0	**0**	**10**	0	0	0	0	0	0	0	0	0	0
Onobrychis viciifolia	Mixture	0	0	0	0	0	0	**10**	**0**	0	0	0	0	0	0	0	0
	Monoculture	0	0	0	0	0	0	**0**	**10**	0	0	0	0	0	0	0	0
Plantago lanceolata	Mixture	0	0	0	0	0	0	0	0	**10**	**0**	0	0	0	0	0	0
	Monoculture	0	0	0	0	0	0	0	0	**0**	**10**	0	0	0	0	0	0
Poa pratensis	Mixture	0	0	0	0	0	0	0	0	0	0	**10**	**0**	0	0	0	0
	Monoculture	0	0	0	0	0	0	0	0	0	0	**0**	**10**	0	0	0	0
Prunella vulgaris	Mixture	0	0	0	0	0	0	0	0	0	0	0	0	**10**	**0**	0	0
	Monoculture	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**10**	0	0
Trifolium repens	Mixture	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**10**	**0**
	Monoculture	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**10**
Crepis biennis	Mixture	**10**	**0**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Monoculture	**1**	**9**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Festuca pratensis	Mixture	0	0	**10**	**0**	0	0	0	0	0	0	0	0	0	0	0	0
	Monoculture	0	0	**0**	**10**	0	0	0	0	0	0	0	0	0	0	0	0
Galium mollugo	Mixture	0	0	0	0	**10**	**0**	0	0	0	0	0	0	0	0	0	0
	Monoculture	0	0	0	0	**0**	**10**	0	0	0	0	0	0	0	0	0	0
Onobrychis viciifolia	Mixture	0	0	0	0	0	0	**10**	**0**	0	0	0	0	0	0	0	0
	Monoculture	0	0	0	0	0	0	**0**	**10**	0	0	0	0	0	0	0	0
Plantago lanceolata	Mixture	0	0	0	0	0	0	0	0	**10**	**0**	0	0	0	0	0	0
	Monoculture	0	0	0	0	0	0	0	0	**0**	**10**	0	0	0	0	0	0
Poa pratensis	Mixture	0	0	0	0	0	0	0	0	0	0	**10**	**0**	0	0	0	0
	Monoculture	0	0	0	0	0	0	0	0	0	0	**0**	**10**	0	0	0	0
Prunella vulgaris	Mixture	0	0	0	0	0	0	0	0	0	0	0	0	**10**	**0**	0	0
	Monoculture	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**10**	0	0
Trifolium repens	Mixture	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**10**	**0**
	Monoculture	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**10**

Species		*Crepis biennis*		*Festuca pratensis*		*Galium mollugo*		*Onobrychis viciifolia*		*Plantago lanceolata*		*Poa pratensis*		*Prunella vulgaris*		*Trifolium repens*	
	History	Mixture	Monoculture	Mixture	Monoculture	Mixture	Monoculture	Mixture	Monoculture	Mixture	Monoculture	Mixture	Monoculture	Mixture	Monoculture	Mixture	Monoculture
Crepis biennis	Mixture	**9**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Monoculture	**0**	**10**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Festuca pratensis	Mixture	0	0	**10**	**0**	0	0	0	0	0	0	0	0	0	0	0	0
	Monoculture	0	0	**0**	**10**	0	0	0	0	0	0	0	0	0	0	0	0
Galium mollugo	Mixture	0	0	0	0	**10**	**0**	0	0	0	0	0	0	0	0	0	0
	Monoculture	0	0	0	0	**0**	**10**	0	0	0	0	0	0	0	0	0	0
Onobrychis viciifolia	Mixture	0	0	0	0	0	0	**10**	**0**	0	0	0	0	0	0	0	0
	Monoculture	0	0	0	0	0	0	**0**	**10**	0	0	0	0	0	0	0	0
Plantago lanceolata	Mixture	0	0	0	0	0	0	0	0	**10**	**0**	0	0	0	0	0	0
	Monoculture	0	0	0	0	0	0	0	0	**0**	**10**	0	0	0	0	0	0
Poa pratensis	Mixture	0	0	0	0	0	0	0	0	0	0	**10**	**0**	0	0	0	0
	Monoculture	0	0	0	0	0	0	0	0	0	0	**0**	**10**	0	0	0	0
Prunella vulgaris	Mixture	0	0	0	0	0	0	0	0	0	0	0	0	**10**	**0**	0	0
	Monoculture	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**10**	0	0
Trifolium repens	Mixture	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**10**	**0**
	Monoculture	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**10**
Crepis biennis	Mixture	**9**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Monoculture	**1**	**8**	0	0	0	0	0	0	0	0	0	0	0	0	0	*1*
Festuca pratensis	Mixture	0	0	**8**	**2**	0	0	0	0	0	0	0	0	0	0	0	0
	Monoculture	0	0	**2**	**8**	0	0	0	0	0	0	0	0	0	0	0	0
Galium mollugo	Mixture	0	0	0	0	**10**	**0**	0	0	0	0	0	0	0	0	0	0
	Monoculture	0	0	0	0	**2**	**7**	0	0	0	0	0	0	*1*	0	0	0
Onobrychis viciifolia	Mixture	0	0	*1*	0	0	0	**7**	**2**	0	0	0	0	0	0	0	0
	Monoculture	0	0	0	0	0	0	**0**	**10**	0	0	0	0	0	0	0	0
Plantago lanceolata	Mixture	0	0	0	0	0	0	*1*	0	**9**	**0**	0	0	0	0	0	0
	Monoculture	0	0	0	0	0	0	0	0	**0**	**10**	0	0	0	0	0	0
Poa pratensis	Mixture	0	0	0	0	0	0	0	0	0	0	**8**	**2**	0	0	0	0
	Monoculture	0	0	0	0	0	0	0	0	0	0	**1**	**9**	0	0	0	0
Prunella vulgaris	Mixture	0	0	0	0	0	0	0	0	0	0	0	0	**9**	**1**	0	0
	Monoculture	0	0	0	0	0	0	0	0	0	0	0	0	**2**	**8**	0	0
Trifolium repens	Mixture	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**6**	**4**
	Monoculture	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**10**

Figure S1: Mean FTIR spectra (n = 10 plant individuals for each curve) for eight European grassland species selected in monocultures or mixtures over 8 years in a biodiversity experiment in Jena, Germany.

Figure S2: Dendrogram of individuals of six species selected in monocultures or mixtures over 8 years in a biodiversity experiment in Jena, Germany, based on values of the second derivative of FTIR spectra (dendrograms for the other two species are presented in Fig. 3 of the main text).

Figure S1

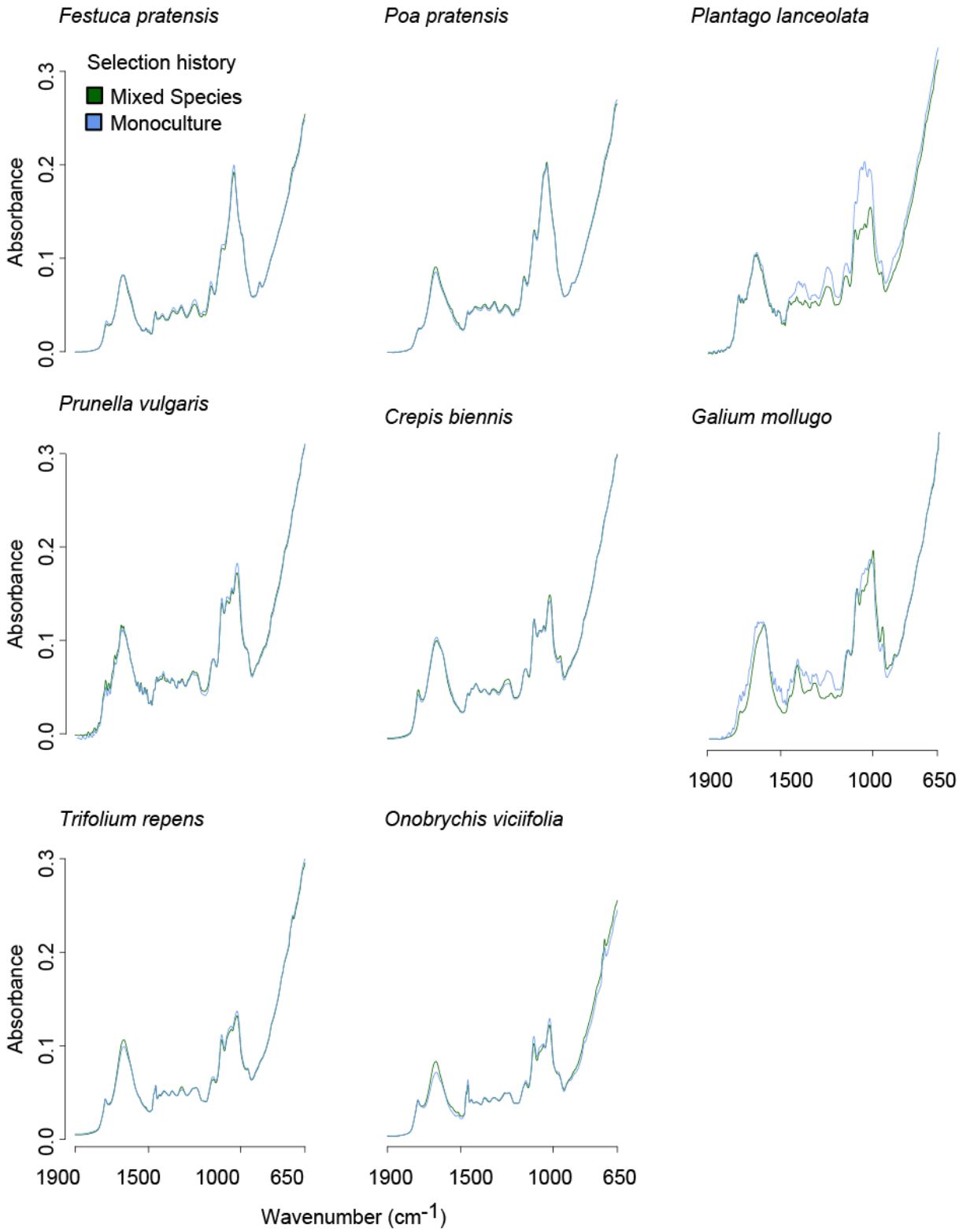
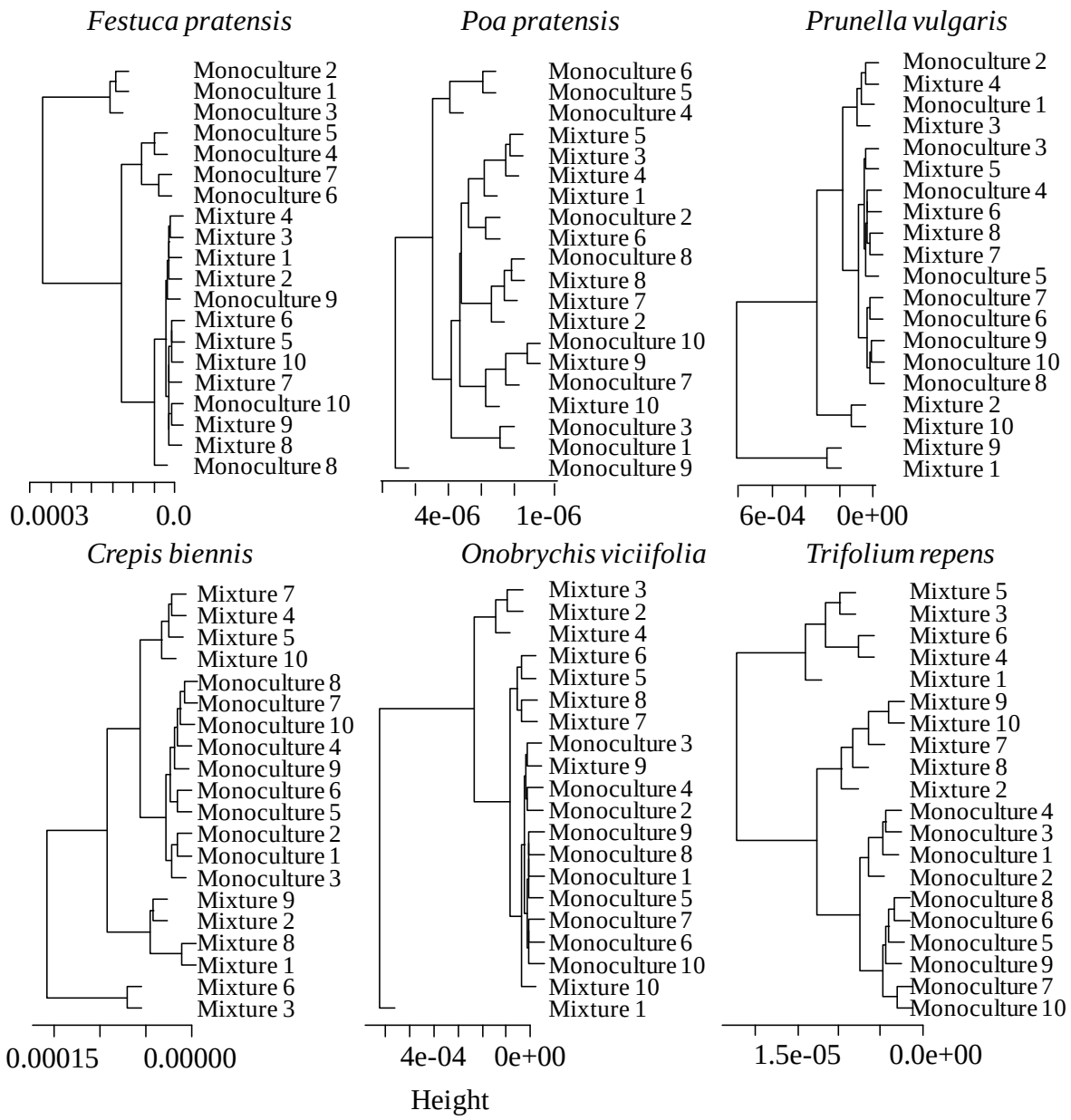

Festuca pratensis
Poa pratensis
Plantago lanceolata
Prunella vulgaris
Crepis biennis
Galium mollugo
Trifolium repens
Onobrychis viciifolia
Selection history
Mixed Species
Monoculture
Absorbance
0.0
0.1
0.2
0.3
1900
1500
1000
650
Wavenumber (cm-1)

Figure S2


Festuca pratensis
Monoculture 2
Monoculture 1
Monoculture 3
Monoculture 5
Monoculture 4
Monoculture 7
Monoculture 6
Mixture 4
Mixture 3
Mixture 1
Mixture 2
Monoculture 9
Mixture 6
Mixture 5
Mixture 10
Mixture 7
Monoculture 10
Mixture 9
Mixture 8
Monoculture 8
0.0003
0.0
Poa pratensis
Monoculture 6
Monoculture 5
Monoculture 4
Mixture 5
Mixture 3
Mixture 4
Mixture 1
Monoculture 2
Mixture 6
Monoculture 8
Mixture 8
Mixture 7
Mixture 2
Monoculture 10
Mixture 9
Monoculture 7
Mixture 10
Monoculture 3
Monoculture 1
Monoculture 9
4e-06
1e-06
Prunella vulgaris
Monoculture 2
Mixture 4
Monoculture 1
Mixture 3
Monoculture 3
Mixture 5
Monoculture 4
Mixture 6
Mixture 8
Mixture 7
Monoculture 5
Monoculture 7
Monoculture 6
Monoculture 9
Monoculture 10
Monoculture 8
Mixture 2
Mixture 10
Mixture 9
Mixture 1
6e-04
0e+00
Crepis biennis
Mixture 7
Mixture 4
Mixture 5
Mixture 10
Monoculture 8
Monoculture 7
Monoculture 10
Monoculture 4
Monoculture 9
Monoculture 6
Monoculture 5
Monoculture 2
Monoculture 1
Monoculture 3
Mixture 9
Mixture 2
Mixture 8
Mixture 1
Mixture 6
Mixture 3
0.00015
0.00000
Onobrychis viciifolia
Mixture 3
Mixture 2
Mixture 4
Mixture 6
Mixture 5
Mixture 8
Mixture 7
Monoculture 3
Mixture 9
Monoculture 4
Monoculture 2
Monoculture 9
Monoculture 8
Monoculture 1
Monoculture 5
Monoculture 7
Monoculture 6
Monoculture 10
Mixture 10
Mixture 1
4e-04
0e+00
Trifolium repens
Mixture 5
Mixture 3
Mixture 6
Mixture 4
Mixture 1
Mixture 9
Mixture 10
Mixture 7
Mixture 8
Mixture 2
Monoculture 4
Monoculture 3
Monoculture 1
Monoculture 2
Monoculture 8
Monoculture 6
Monoculture 5
Monoculture 9
Monoculture 7
Monoculture 10
1.5e-05
0.0e+00
Height

Discussion

General Discussion

The results of biodiversity research of the last two decades points unequivocally to the importance of maintaining high levels of biodiversity to ensure levels and stability of the ecosystem processes that support human health and well-being (Hooper et al. 2005, Cardinale et al. 2012, Naeem et al. 2012, Reich et al. 2012). Indeed, experimental evidence suggests that the loss of even a few species from a highly diverse community may have an increasingly negative impact on ecosystem functioning over time (Cardinale et al. 2012, Reich et al. 2012). The major drivers of species loss are increasing anthropogenic activities (Naeem et al. 2012), with loss of habitat considered to be the greatest cause of species loss from an ecosystem (Barbault and Sastrapradja 1995). This can occur by the introduction of new (invasive) species (Radosevich et al. 2007), homogenization of species pools (Naeem et al. 2012) and in general human population growth (McKee et al. 2004). Habitat loss may restrict the ability of a species to adapt to predicted climate change, with fragmentation and habitat reduction limiting gene flow and migration (Thuiller et al. 2011, Morueta-Holme et al. 2013, Pfeifer-Meister et al. 2013). Additionally, plant species ranges typically move towards higher latitudes and altitudes with changes in the climate (Engelkes et al. 2008, Beaumont et al. 2009). This has implications for the future species richness of plant communities (Callaway and Aschehoug 2000, Liao et al. 2008) and ecosystem functionality (Steinbeiss et al. 2008, Maestre et al. 2012, Naeem et al. 2012). Understanding the mechanisms that maintain plant species coexistence and the potential effects of species loss is therefore a critically important direction in ecological research.

The focus of this dissertation was to test novel hypotheses about mechanisms for the observed positive temporal effect of grassland biodiversity on biomass production in experiments. Understanding how primary producers coexist to support and maintain productivity will give us a better knowledge of the potential impact of biodiversity loss in

grassland communities. Specifically I focused on a novel hypothesis that selection for greater combining ability through division of labor with other plant species occurs in mixtures through the selection of genotypes adapted to their biotic environment. Selection for division of labor implies that in mixtures plant individuals would be selected for reduced competition with other species in the community and therefore a greater dissimilarity to allow for greater resource complementarity. For example, in mixtures, tall-statured species may grow taller to capture overstory light whereas small-statured species may have broader leaves (greater SLA) for increased light acquisition in the understory. Another mechanism for division of labor may arise from greater differentiation in rooting depth in mixtures in contrast to when plant individuals of these species are grown in monoculture communities. It is conceivable that plant individuals could therefore be selected for reduced competition and increased complementarity in resource capture between species resulting in increased division of labor with a positive effect on the productivity of species mixtures as observed in this study.

I consistently found in my experiments (Chapters 1 and 2) that productivity was greater in mixtures than in monocultures. This confirms previous results which demonstrated that even slight increases in species diversity in grasslands can lead to an increase in biomass production (Kirwan et al. 2007). I demonstrated that selection for mixture types and monoculture types appears to occur in experimental grassland communities (Chapter 1). Mixture types are plant individuals that perform better when planted in mixtures in contrast to monoculture types, which perform better in monocultures. I showed that increasing complementarity effects are enhanced by plant–soil feedbacks and by selection of plants adapted to mixture communities (Chapter 2). The data presented also demonstrate that alterations in metabolite composition occurred in plant individuals selected in monoculture vs. mixture (Chapter 3). The results of this study indicate that species diversity may influence selection processes within plant communities. This provides new insights into plant coexistence and deepens our understanding of the impact of biodiversity loss not only on

primary productivity and ecosystem functioning, but also the co-adaptive evolutionary trajectories of species within diverse grasslands.

Selection for Monoculture and Mixture Types

Niche differences can increase the strength of intraspecific competition compared to interspecific competition (Amarasekare 2003). This implies that plant individuals in communities of differing species diversity are exposed to different selection pressure. Furthermore, the diversity of species within a community may play an important part in shaping selection pressure within each population (Vellend and Geber 2005). This is consistent with a competitive combining ability hypothesis whereby local evolutionary changes arise from selection through competition (Taylor and Aarssen 1990). The difference in selection processes in mixtures and monocultures has been demonstrated in a field experiment focusing on four grassland species which showed that strong intraspecific competition was more negative than interspecific competition in competitive effect and response (Farrer and Goldberg 2011). Further evidence of such selection processes was found in a study focusing on the interactions of the legume *Trifolium repens* in competition with commonly co-occurring grasses (Turkington and Harper 1979, Turkington 1989). Similarly, Lüscher et al. (1992) found that growing *Trifolium repens* with its natural neighbor *Lolium perenne* had a positive effect on productivity. Therefore there is strong evidence to suggest different selection processes occur in plant monocultures and mixtures; and these may lead to differential evolution of plant monoculture and mixture types.

Productivity increased in experimental test mixtures of plant types selected in mixtures for eight years in contrast to types selected in monoculture. In Chapter one, I was thus able to demonstrate selection for increased interspecific combining ability in mixed grassland communities through division of labor, a result which in part may explain the observed increase in biodiversity effects over time in grassland experiments (Reich et al. 2012). This

increase in division of labor for mixture types in test mixtures was independent of species or functional group identity and thus appears to be a general phenomenon for these grassland species. In addition, we found that plant individuals selected in monoculture communities produced a higher yield than mixture types when replanted in monoculture test communities, suggesting there was also a selection for monoculture types over the 8-year time frame (Chapter 1).

I speculate that the positive effect on biodiversity effects of selection in mixture will continue to increase with time, which could be tested by repeating our study after an additional time period. Additionally, the consequence of adaption to the total local biotic environment needs to be considered, such as the beneficial and antagonistic interactions with above- and belowground organisms. If plant populations in mixed grassland communities change over time to function optimally in species-rich environments, then the long-term impact of species loss through homogenization, urbanization and invasion may be more far-reaching than just an initial decrease in ecosystem functioning.

Trait Differentiation in Monocultures vs. Mixed-Species Plant Communities

In a first step to unraveling the complex underlying mechanisms of selection for increased complementarity in mixtures, we included trait measurements to give some indication of resource-use and phenological complementarity (plant height, specific leaf area [SLA], biomass; Chapter 1). Previously plant diversity has been found to influence traits of grass species that are generally associated with light and nitrogen acquisition, such as increases in shoot, leaf and stem length (Gubsch et al. 2011). In our study, mixture types showed a greater difference between species in height and SLA than did monoculture types. This is consistent with evidence from a study focusing on legumes showing that trait variation associated with light and nutrient capture in grassland species can be attributed to an increase in plant diversity (Roscher et al. 2011). One of the underlying mechanisms explaining

increased performance of mixture types planted in mixture communities in our study may therefore be increased trait differences between species. Such an increase in trait differences is indicative of selection for increased division of labor within mixed-species plant communities.

The Role of Functional Groups

The interspecific combining ability observed in Chapter 1 appeared to be a general effect and was not dependent on the particular functional group combination within the experimental communities or the species combination nested within these functional group combinations. Given that some authors have argued that ad-hoc functional groups may have limited utility in understanding community dynamics (Petchey et al. 2004, Wright et al. 2006), the weak effects of our pre-defined functional groups on selection for mixture types is not entirely surprising. However, some groupings of plants are clearly important, as the grasses all have similar rooting structure and growth form, and legumes certainly have a strong and obviously distinct effect on communities via their mutualisms with nitrogen-fixing rhizobia (Spehn et al. 2002). Indeed, there were tendencies for varying responses of plant monoculture and mixture types when grown in test mixtures of varying functional group combinations. I found that biodiversity effects in test communities assembled from mixture types were strongest for grass–grass combinations and weakest for grass–legume combinations. Karan et al. (2007) found that positive interactions between grasses, legumes and grass–legume pairs were similarly strong in mixtures and contributed equally to increased biomass production in mixtures. The weak response of grass–legume combinations in my experiment is surprising considering that there is substantial evidence that this functional group combination generally shows particularly strong complementarity effects (Hector et al. 1999, Spehn et al. 2005). The poor response of grass–legume species combinations to selection for mixture types in our study may be explained by the fact that this combination probably reflects an old co-evolutionary combination (Bremer et al. 1998) in which plants

have already been strongly selected for mixture performance and no further selection was therefore possible.

The Role of Plant–Soil Feedbacks

Species diversity in plant communities has been attributed in part to the direction and strength of plant–soil feedbacks and the contribution of such feedbacks to the competitive strength of plant species within the community (Revilla et al. 2013). Plant–soil feedbacks can therefore influence the competitive interactions between plants (Callaway et al. 2004; Klironomos 2002). Using the data collected in Chapter 2 it was possible to demonstrate plant–soil feedbacks increased complementarity (Eisenhauer 2012, Kulmatiski et al. 2012) among the plants in our study; particularly for plants of mixture selection history. Soil feedbacks in mixtures could reduce niche overlap between species by increasing complementarity among species and thereby increasing primary productivity for the community. For instance, the resource niche of a plant can be either expanded or shrunk by mutualistic mycorrhizal fungi (Klironomos et al. 2000). The competitive balance between plant species within such a community can therefore be altered (Scheublin et al. 2007, Collins and Foster 2009). This suggests that the feedback effects given the common 8-year history between the plants and associated soil biota may have contributed to selection pressure in mixtures for increased combining ability.

Negative plant–soil feedbacks are diluted in mixed-species communities (Kulmatiski et al. 2012). In contrast, species-specific plant pathogens can commonly accumulate under high-density monocultures (Schnitzer et al. 2011). Plants selected in monoculture communities may therefore be better defended against pathogens relative to mixture plant types. For example, Lipowsky and colleagues (2011) observed in their reciprocal transplant experiment that four out of five species showed increased herbivory defense in plants that had been selected in monoculture as opposed to mixed-species plant communities, suggesting

there may be a tradeoff between growth and defense. Increased defense against pathogens does occur in agriculture. For example, yields of wheat and barley monocultures decline as a result of soil borne pathogens over an initial time period but then recover with the development of increased defenses against these pathogens (Cook and Weller 2004). Moreover, the impact of a fungal root disease associated with wheat monocultures is reduced once the wheat plants become colonized with soil bacteria that increase the wheat's resistance to the fungal disease (Weller et al. 2002). Selection pressure for increased resistance to pathogens may therefore conceivably explain the increased yield we observed in monoculture types assembled in monoculture test communities (Chapter 1).

The growth–defense tradeoff hypothesis suggesting that individuals selected in monoculture grassland communities develop increased resistance to pathogens is currently being tested in an exploratory study with the intention to expand the study into a PhD project. For the exploratory study, cuttings were collected in spring 2013 from monoculture and mixed-species communities in the Jena Experiment, three years on from the present study. These cuttings were propagated and then grown as individuals in four soil treatments which attempted to separate positive and negative soil feedbacks. The aim of this study was to test whether monoculture types are selected for increased defense against pathogens and whether mixture types lose this defense in a tradeoff for increased growth in mixtures.

Phenotypes or Genotypes within Species?

The process by which differentiation into monoculture types and mixture types occurs within these plant communities is unclear. There may be a sorting out of genotypes, pre-adapted to monocultures vs. mixtures, from a large initial variation in the sown seed populations of the test species over time in monoculture vs. mixed-species communities. Sorting out of genotypes may enhance species coexistence (Vellend and Geber 2005) as had been found in a study on mixtures of sedge and grass species in which certain genotypes were

better able to coexist with interspecific neighbors (and positively influenced plant performance) than with other genotypes in the experiment (Fridley et al. 2007). In the present study plants selected in mixtures showed greater differences in traits in contrast to monoculture types (Chapter 1) and significant differences were observed between the metabolic fingerprints of monoculture and mixture types (Chapter 3). These phenotypic differences were observed in plants raised from cuttings or seedlings under common environmental conditions and thus must have hereditary causes. These are likely genotypic, but epigenetic or maternal carry-over causes can also not be ruled out.

Some of the selection effects on traits were clearer for cuttings. Underlying this result could be that seedlings had at least one recombination event during the selection history of mixture and monoculture types planted in our test communities. Thorpe et al. (2011) suggested that plants that are adapted to compete with one species or genotype may lose the same ability to compete with other species or genotypes. This could partially explain the decreased productivity of mixture types planted into monoculture test communities and vice versa. In addition, epigenetic variation and persistent maternal carry-over effects might have contributed to our results. Maternal effects can allow for plant adaptation in natural settings (Galloway and Etterson 2007) which can be transferred as an inherited environmental effect to offspring (Roach and Wulff 1987, Rossiter 1996). Furthermore, heritable changes could occur through epigenetically changed gene expression in response to plant species diversity.

To determine whether there was an initial sorting out of genotypes in monoculture vs. mixed-species communities, samples of the original seed material used to sow the Jena experiment should be compared with the genotypes currently occurring in the field plots. This research question will be examined further in a master project. In a doctoral study planned for 2014/15, plant material collected from the Jena experiment will be studied to determine whether selection occurs at a phenotypic or genotypic level.

Biodiversity Increases Resistance against Weeds in Mixed-Species Plant Communities

Higher species diversity in plant communities has been associated with increased resistance to invasion (Levine 2000, Naeem et al. 2000, Kennedy et al. 2002, MacDougall et al. 2009, Turnbull et al. 2010).Test communities of plants with mixed species selection history, in Chapter 2, had fewer invasives than test communities with monoculture-selection history. These results imply that the age of grassland plant communities may play a role in resistance against invasion (Chapter 2). Furthermore, increased complementarity effects in perennial grasslands have been suggested as the mechanism to inhibit invasion (Jiang et al. 2007). I found increased complementarity effects for mixture types (Chapter 1, 2), implying that complementarity between the species in this study may have contributed to the observed increased resistance to invasion. Because one of the greater causal agents for habitat loss for native plant species is the invasion by exotic plants (Gurevitch and Padilla 2004), understanding how plant communities can resist invasion is critical to reducing the threat these exotics pose to such natural communities (Davis 2003). By increasing the resistance of plant communities to invasive plants through increased biodiversity (Blumenthal et al. 2005) and community age (results of this study) the spread of invasive species into new habitats may be controlled.

Conclusion

The results of this thesis provide new insights into the mechanisms governing the temporal influence of plant species diversity on biodiversity effects. I demonstrated that the diversity of the local plant species community can select for mixture and monoculture plant types over eight years in a biodiversity experiment. Specifically, I was able to demonstrate that complementarity effects increased for plants that had undergone eight years of selection in mixtures and such effects increased in the presence of a co-evolved soil community. In contrast, plants selected in monocultures performed best if planted in monocultures and appeared to be selected for increased defense against pathogens.

The significance of understanding how diversity positively influences ecosystem functioning, such as indicated by primary productivity, is an important contribution towards our understanding of fundamental issues in plant ecology. Such an understanding is essential for future biodiversity conservation in the face of increasing pressure from human impacts and for maintaining ecosystem functions that may have direct human benefits. For example, fodder production, carbon sequestration and resistance to invasion are all increased by plant species diversity in grasslands (Naeem et al. 2012). Increased productivity and compositional stability of grassland communities may also enhance carbon sequestration and had been proposed as a method of partly mitigating the problem of greenhouse emissions of livestock (Soussana et al. 2010), emphasizing the relevance of the results of this study.

I made the novel finding that plant individuals within monoculture and mixture communities may be sent on different evolutionary trajectories over time through coadaptation to selection processes occurring within such communities. As biodiversity conservation is not only focused on maintaining species diversity but also genotypic diversity within species, such a result has serious implications. The loss of genotypic diversity implies that, within plant species, individuals with the ability to productively coexist with other species will diminish. Despite increased productivity of monoculture types in monocultures, such monocultures are less productive than mixtures composed of mixture types as I observed in this study. Loss of species and genotypes that perform well in mixtures suggests therefore a growing loss of primary productivity with negative implications across all trophic levels.

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Appendix

Poster

Eight years of selection leads to differentiation into monoculture and mixture types in grasslands

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Why does productivity in grasslands increase over time?

Historically, plant species co-existence has been interpreted as:

- **Niche segregation increasing complementary resource use.**
- **Pathogen regulation of specific plant species.**

Monoculture grassland

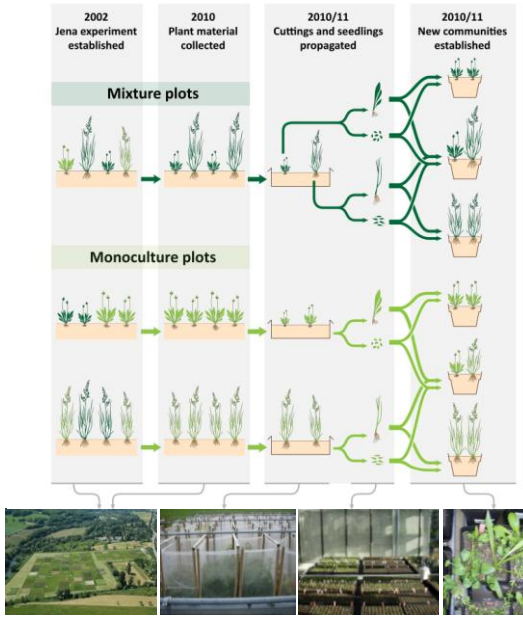
Mixed species grassland

Objective

To test the novel hypothesis of selection for the biotic environment, using an eight year grassland biodiversity experiment, we predict:

- **Mixture types have been selected for combining ability, with plants from mixed species plots more productive than plants from monoculture plots when replanted in experimental mixtures.**
- **Monoculture types have been selected for increased pathogen defense, with plants from monoculture plots more productive than plants from mixed species plots when replanted in experimental monocultures.**

Methods



Results

Selection history influences productivity in experimental communities.

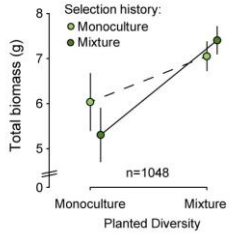


Fig. 1: Aboveground biomass (± 1 s.e.m.) indicating the difference in slopes between plants selected for in monoculture and mixture history communities planted in monocultures and mixtures.

Selection for increased complementarity in mixture types.

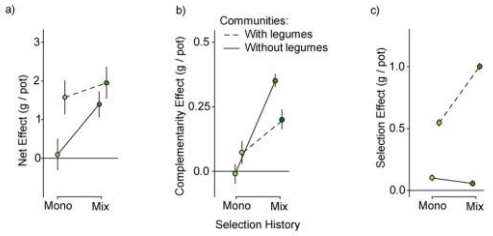


Fig. 2: a) Net biodiversity effect [1] partitioned into b) complementarity effects and c) selection effects.

Increased differences in traits between species selected for in mixtures.

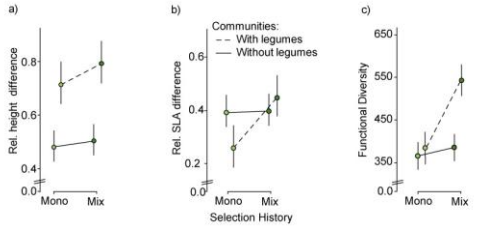


Fig. 3: Relative difference for key plant traits, a) height, b) SLA by values between species for each selection history, c) Functional Diversity [2] calculated by trait values of height, SLA and reproductive biomass within species by selection history.

Conclusions

- **First indication of selection for increased combining ability indicated by greater complementarity in mixed species plots after 8 years possibly explaining the increase in productivity over time.**
- **The increase in complementarity for mixture types seems to result from increased trait differences between species in mixtures.**
- **Increased productivity of monoculture types in monocultures possibly due to selection for increased pathogen defense.**

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Acknowledgements

SNF grant: 130720, Matthias Furler, Daniel Trujillo Villegas, Theres Zwimpfer, Leigh Oesch, René Husi, the gardeners of the Jena Experiment.

Acknowledgements

Acknowledgements

Thank you to Bernhard Schmid for his constant enthusiastic input, his patient instruction in the quagmire of mixed models and for almost always having “five minutes” (usually turning into 2 hours). Thank you to Dan Flynn for his generous help and helping me become a fledgling “R ninja”. Thank you to my committee members, Andy Hector, Gerlinde de Deyn, Pascal Niklaus and Alex Weigelt, for their advice and time investment. To Daniel Trujillo, Leigh Oesch and student helpers, thanks for all the practical help and support in the greenhouse and field work. To Matthias Furler, thanks for all his advice and practical help with the graphics. To Isabel Schöchli and Theres Zwimpfer, thanks for their administrative support and gardening advice respectively. To my fellow PhD students, thanks for their constant encouragement and really good company. All the people at the Institute, thank you.

For my parents, Heather and Peter Dingley, and siblings; thanks for their moral support and their faith in me that I could achieve my goals. Gerrilie van Breda, thanks for her help with my children particularly in the first few years when looking after my plants demanded so much of my attention. To my husband, Daniel, thanks for supporting me in my career. And last but most important to my children, Tamsyn and Caitlyn, for their support, patience with all the overtime hours and their practical help, my overwhelming gratitude.